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(54) Title: GLYCOGEN SYNTHASE KINASE-3 INHIBITORS

(57) Abstract: Compounds capable of inhibiting GSK-3 activity, pharmaceutical compositions including same and methods of using same in the treatment of GSK-3 mediated conditions are disclosed.

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GLYCOGEN SYNTHASE KINASE-3 INHIBITORSFIELD AND BACKGROUND OF THE INVENTION

5 The present invention relates to novel compounds for inhibiting glycogen synthase kinase-3 (GSK-3) and their use in regulating biological conditions mediated by GSK-3 activity and, more particularly, to the use of these compounds in the treatment of biological conditions such as type II diabetes, neurodegenerative disorders and diseases and affective disorders.

10 Protein kinases, the enzymes that phosphorylate protein substrates, are key players in the signaling of extracellular events to the cytoplasm and the nucleus, and take part in practically any event relating to the life and death of cells, including mitosis, differentiation and apoptosis. As such, protein kinases have long been favorable drug targets. However, since the activity of protein kinases is crucial to the
15 well being of the cell, while their inhibition oftentimes leads to cell death, their use as drug targets is limited. Although cell death is a desirable effect for anticancer drugs, it is a major drawback for most other therapeutics.

 Glycogen synthase kinase-3 (GSK-3), a member of the protein kinases family, is a cytoplasmic proline-directed serine-threonine kinase that is involved in insulin
20 signaling and metabolic regulation, as well as in Wnt signaling and the scheme of cell fate during embryonic development. Two similar isoforms of the enzyme, termed GSK-3 α and GSK-3 β , have been identified.

 GSK-3 has long been considered as a favorable drug target among the protein kinase family since unlike other protein kinases, which are typically activated by
25 signaling pathways, GSK-3 is normally activated in resting cells, and its activity is attenuated by the activation of certain signaling pathways such as those generated by the binding of insulin to its cell-surface receptor. Activation of the insulin receptor leads to the activation of protein kinase B (PKB, also called Akt), which in turn phosphorylates GSK-3, thereby inactivating it. The inhibition of GSK-3 presumably
30 leads to the activation of glycogen synthesis. The intricate insulin-signaling pathway is further complicated by negative-feedback regulation of insulin signaling by GSK-3 itself, which phosphorylates insulin-receptor substrate-1 on serine residues (Eldar-Finkelman et al., 1997).

Therefore, synthetic GSK-3 inhibitors might mimic the action of certain hormones and growth factors, such as insulin, which use the GSK-3 pathway. In certain pathological situations, this scheme might permit the bypassing of a defective receptor, or another faulty component of the signaling machinery, such that the biological signal will take effect even when some upstream players of the signaling cascade are at fault, as in non-insulin-dependent type II diabetes.

The regulation of glycogen catabolism in cells is a critical biological function that involves a complex array of signaling elements, including the hormone insulin. Through a variety of mediators, insulin exerts its regulatory effect by increasing the synthesis of glycogen by glycogen synthase (GS). A key event in insulin action is the phosphorylation of insulin receptor substrates (IRS-1, IRS-2) on multiple-tyrosine residues, which results in simultaneous activation of several signaling components, including PI3 kinase (Myers et al, 1992)). Similarly, the activity of glycogen synthase is suppressed by its phosphorylation. There is a marked decrease in glycogen synthase activity and in glycogen levels in muscle of type II diabetes patients (Damsbo et al., 1991; Nikoulina et al., 1997; Shulman et al., 1990).

One of the earliest changes associated with the onset of type II (non-insulin dependent) diabetes is insulin resistance. Insulin resistance is characterized by hyperinsulemia and hyperglycemia. Although the precise molecular mechanism underlying insulin resistance is unknown, defects in downstream components of the insulin signaling pathway are considered to be the cause.

Glycogen synthase kinase-3 (GSK-3) is one of the downstream components of insulin signaling. It was found that high activity of GSK-3 impairs insulin action in intact cells, by phosphorylating the insulin receptor substrate-1 (IRS-1) serine residues (Eldar-Finkelman et al, 1997), and likewise, that increased GSK-3 activity expressed in cells results in suppression of glycogen synthase activity (Eldar-Finkelman et al, 1996). Further studies conducted in this respect uncovered that GSK-3 activity is significantly increased in epididymal fat tissue of diabetic mice (Eldar-Finkelman et al, 1999). Subsequently, increased GSK-3 activity was detected in skeletal muscle of type II diabetes patients (Nickoulina et al, 2000). Additional recent studies further established the role of GSK-3 in glycogen metabolism and insulin signaling (for review see, Eldar-Finkelman, 2002; Grimes and Jope, 2001;

Woodgett, 2001), thereby suggesting that the inhibition of GSK-3 activity may represent a way to increase insulin activity *in vivo*.

GSK-3 is also considered to be an important player in the pathogenesis of Alzheimer's disease. GSK-3 was identified as one of the kinases that phosphorylate tau, a microtubule-associated protein, which is responsible for the formation of paired helical filaments (PHF), an early characteristic of Alzheimer's disease. Apparently, abnormal hyperphosphorylation of tau is the cause for destabilization of microtubules and PHF formation. Despite the fact that several protein kinases were shown to promote phosphorylation of tau, it was found that only GSK-3 phosphorylation directly affected tau ability to promote microtubule self-assembly (Hanger et al., 1992; Mandelkow et al., 1992; Mulot et al., 1994; Mulot et al., 1995). Further evidence for the GSK-3 role in this respect came from studies of cells overexpressing GSK-3 and from transgenic mice that specifically expressed GSK-3 in brain. In both cases GSK-3 led to generation of the PHF like epitope tau (Lucas et al., 2001).

GSK-3 is further linked with Alzheimer's disease by its role in cell apoptosis. The fact that insulin is a survival factor of neurons (Barber et al., 2001) and initiates its anti-apoptotic action through activation of PI3 kinase and PKB (Barber et al., 2001), suggested that GSK-3, which is negatively regulated by these signaling components, promotes neuronal apoptosis. Several studies have indeed confirmed this view, and showed that GSK-3 is critically important in life and death decision. Furthermore, its apoptotic function was shown to be independent of PI3 kinase. Overexpression of GSK-3 in PC12 cells caused apoptosis (Pap et al., 1998). Activation of GSK-3 in cerebellar granule neurons mediated migration and cell death (Tong et al., 2001). In human neuroblastoma SH-SY5Y cells, over expression of GSK-3 facilitated staurosporine-induced cell apoptosis (Bijur et al., 2000).

The relation between GSK-3 inhibition and the prevention of cells death has been further demonstrated by studies showing that expression of Frat1, a GSK-3 β inhibitor, was sufficient to rescue neurons from death induced by inhibition of PI3 kinase (Crowder et al., 2000).

Another implication of GSK-3 was detected in the context of affective disorders, i.e., bipolar disorders and manic depression. This linkage was based on the findings that lithium, a primary mood stabilizer frequently used in bipolar disease, is a strong and specific inhibitor of GSK-3 at the therapeutic concentration range used in

clinics (Klein et al., 1996; Stambolic et al., 1996; Phiel et al., 2001). This discovery has led to a series of studies that were undertaken to determine if lithium could mimic loss of GSK-3 activity in cellular processes. Indeed, lithium was shown to cause activation of glycogen synthesis (Cheng et al., 1983), stabilization and accumulation of β -catenin (Stambolic et al., 1996), induction of axis duplication in *Xenopus* embryo (Klein et al., 1996), and protection of neuronal death (Bijur et al., 2000). Valproic acid, another commonly used mood stabilizer has also been found to be an effective GSK-3 inhibitor (Chen et al., 1999). Altogether, these studies indicated that GSK-3 is a major *in vivo* target of lithium and valproic acid and thus has important implications in novel therapeutic treatment of affective disorders.

One mechanism by which lithium and other GSK-3 inhibitors may act to treat bipolar disorder is to increase the survival of neurons subjected to aberrantly high levels of excitation induced by the neurotransmitter, glutamate (Nonaka et al., 1998). Glutamate-induced neuronal excitotoxicity is also believed to be a major cause of neurodegeneration associated with acute damage, such as in cerebral ischemia, traumatic brain injury and bacterial infection. Furthermore, it is believed that excessive glutamate signaling is a factor in the chronic neuronal damage seen in diseases such as Alzheimer's, Huntington's, Parkinson's, AIDS associated dementia, amyotrophic lateral sclerosis (AML) and multiple sclerosis (MS) (Thomas, 1995).

Consequently, GSK-3 inhibitors are believed to be a useful treatment in these and other neurodegenerative disorders. Indeed, dysregulation of GSK-3 activity has been recently implicated in several CNS disorders and neurodegenerative diseases, including schizophrenia (Beasley et al., 2001; Kozlovsky et al., 2002), stroke, and Alzheimer's disease (AD) (Bhat and Budd, 2002; Hernandez et al., 2002; Lucas et al., 2001; Mandelkow et al., 1992).

Recent work has further demonstrated that GSK-3 is involved in additional cellular processes including development (He et al, 1995), oncogenesis (Rubinfeld et al, 1996) and protein synthesis (Welsh et al, 1993). Importantly, GSK-3 plays a negative role in these pathways. This further suggests that GSK-3 is a cellular inhibitor in signaling pathways.

In view of the wide implication of GSK-3 in various signaling pathways, development of specific inhibitors for GSK-3 is considered both promising and important regarding various therapeutic interventions as well as basic research.

As is mentioned above, some mood stabilizers were found to inhibit GSK-3. However, while the inhibition of GSK-3 both by lithium chloride (LiCl) (PCT International patent application WO 97/41854) and by purine inhibitors (PCT International patent application WO 98/16528) has been reported, these inhibitors are not specific for GSK-3. In fact, it was shown that these drugs affect multiple signaling pathways, and inhibit other cellular targets, such as inositol monophosphatase (IMPase) and histone deacetylases (Berridge et al., 1989; Phiel and Klein, 2001).

Similarly, an engineered cAMP response element binding protein (CREB), a known substrate of GSK-3, has been described (Fiol et al, 1994), along with other potential GSK-3 peptide inhibitors (Fiol et al, 1990). However, these substrates also only nominally inhibit GSK-3 activity.

Other GSK-3 inhibitors were recently reported. Two structurally related small molecules SB-216763 and SB-415286 (Glaxo SmithKline Pharmaceutical) that specifically inhibited GSK-3 were developed and were shown to modulate glycogen metabolism and gene transcription as well as to protect against neuronal death induced by reduction in PI3 kinase activity (Cross et al., 2001; Coghlan et al., 2000). Another study indicated that Indurubin, the active ingredient of the traditional Chinese medicine for chronic myelocytic leukemia, is a GSK-3 inhibitor. However, Indirubin also inhibits cyclic-dependent protein kinase-2 (CDK-2) (Damiens et al., 2001). These GSK-3 inhibitors are ATP competitive and were identified by high throughput screening of chemical libraries. It is generally accepted that a major drawback of ATP-competitive inhibitors is their limited specificity (Davies et al., 2000).

A general strategy for developing specific peptide and other GSK-3 inhibitors is reported in WO 01/49709 and in U.S. Patent Application Publication No. 20020147146, by the present inventor, which are incorporated by reference as if fully set forth herein. This general strategy is based on defining the structural features of a GSK-3 substrate, and developing GSK-3 inhibitors in accordance with these features. However, while these publications delineate these structural features and teach various short peptides that efficiently inhibit GSK-3 activity, they fail to teach the design and synthesis of small molecules that could serve as GSK-3 inhibitors. PCT/IL03/01057, by the present inventor, discloses that attaching a hydrophobic moiety to a termini of a peptide GSK-3 inhibitor enhances its inhibition activity.

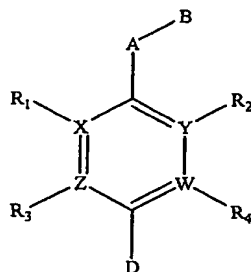
However, although peptides are intriguing drug targets, their use is oftentimes limited by, for example, biological instability, immunogenicity, poor capability to cross biological membranes such as cell membranes and the blood brain barrier (BBB), and the like.

- 5 There is thus a widely recognized need for, and it would be highly advantageous to have, non-peptidic compounds for inhibiting GSK-3 activity, devoid of the above limitations.

SUMMARY OF THE INVENTION

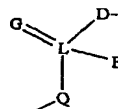
- 10 The present inventor has now surprisingly found that compounds which are designed according to the unique features of the recognition motif of a GSK-3 substrate exhibit substrate competitive inhibition activity toward GSK-3 and can therefore be efficiently used in various applications where reducing the activity of GSK-3 is beneficial.

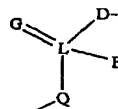
- 15 Hence, according to one aspect of the present invention there is provided a compound having a general formula:



wherein:

- 20 X, Y, Z and W are each independently a carbon atom or a nitrogen atom;
A is alkyl or absent;



B is a negatively charged group having a formula , wherein L is selected from the group consisting of a phosphor atom, a sulfur atom, a silicon atom, a boron atom and a carbon atom; Q, G and D are each independently selected from the group consisting of oxygen and sulfur;; and E is selected from the group consisting of

hydroxy, alkoxy, aryloxy, carbonyl, thiocarbonyl, O-carboxy, thiohydroxy, thioalkoxy and thioaryloxy or absent;

D is selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and a hydrophobic moiety; and

R₁, R₂, R₃ and R₄ are each independently selected from the group consisting of hydrogen, a lone pair of electrons, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanyliinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, and an ammonium ion,

or a pharmaceutically acceptable salt thereof,

provided that at least one of X, Y, Z and W is a nitrogen atom and/or at least one of R₁, R₂, R₃ and R₄ is a group containing at least one amino moiety, and with the proviso that the compound is not pyridoxal phosphate

According to further features in preferred embodiments of the invention described below, D in the above formula is a hydrophobic moiety, and thus, according to another aspect of the present invention there is provided a compound having the formula described above, wherein D is a hydrophobic moiety. The compound according to this aspect of the present invention includes also the compounds excluded above, substituted by the hydrophobic moiety.

The compounds described hereinabove are capable of inhibiting an activity of GSK-3.

According to still further features in the described preferred embodiments A is alkyl.

According to still further features in the described preferred embodiments L is a phosphor atom.

According to still further features in the described preferred embodiments each of Q, G and D is oxygen, and E is preferably hydroxy.

5 According to still further features in the described preferred embodiments at least one of X, Y, Z and W is a nitrogen atom.

According to still further features in the described preferred embodiments at least two of X, Y, Z and W are nitrogen atoms. Preferably either X and Y are each a nitrogen atom or Z and W are each a nitrogen atom.

-10 According to still further features in the described preferred embodiments at least one of R₁, R₂, R₃ and R₄ is a group containing at least one amino moiety.

According to still further features in the described preferred embodiments at least two of R₁, R₂, R₃ and R₄ are groups containing at least one amino moiety. Preferably either each of R₁ and R₂ or each of R₃ and R₄ is a group containing at least
15 one amino moiety.

Examples of groups containing at least one amino moiety include, without limitation, guanidino, guanidinoalkyl, aminoalkyl, analogs thereof, derivatives thereof and any combination thereof.

According to still further features in the described preferred embodiments the
20 group containing at least one amino moiety comprises at least one positively charged group

According to still further features in the described preferred embodiments the positively charged group comprises an ammonium ion. Alternatively, the positively charged group has a chemical structure that is derived from a side chain of a
25 positively charged amino acid, such as, but not limited to, arginine, lysine, histidine, proline and any derivative thereof.

When D is hydrophobic moiety, the hydrophobic moiety is preferably selected from the group consisting of a fatty acid residue, a saturated alkylene chain having between 4 and 30 carbon atoms, an unsaturated alkylene chain having between 4 and
30 30 carbon atoms, an aryl, a cycloalkyl and a hydrophobic peptide sequence.

The fatty acid can be, for example, myristic acid, lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid, linoleic acid or linolenic acid.

Preferred compounds according to the present invention further include compounds in which each of X, Y, Z and W is a carbon atom; and at least one of R₁, R₂, R₃ and R₄ is the group containing at least one amino moiety as described above.

Further preferred compounds are those in which each of X, Y and Z is a carbon atom and W is a nitrogen atom.

According to still another aspect of the present invention there is provided a pharmaceutical composition that comprises, as an active ingredient, a compound as is described hereinabove, which is capable of inhibiting an activity of GSK-3, and a pharmaceutically acceptable carrier.

According to further features in preferred embodiments of the invention described below, the pharmaceutical composition is packaged in a packaging material and is identified in print, on or in the packaging material, for use in the treatment of a biological condition associated with GSK-3 activity, as is detailed hereinbelow.

According to still further features in the described preferred embodiments the pharmaceutical composition further comprises at least one additional active ingredient that is capable of altering an activity of GSK-3, as is detailed hereinbelow.

According to yet another aspect of the present invention there is provided a method of treating a biological condition associated with an activity of GSK-3, which comprises administering to a subject in need thereof a therapeutically effective amount of a compound that is capable of inhibiting an activity of GSK-3, as is described hereinabove.

According to further features in preferred embodiments of the invention described below, the method according to this aspect of the present invention further comprises co-administering to the subject at least one additional active ingredient, which is capable of altering an activity of GSK-3.

The additional active ingredient can be an active ingredient that is capable of inhibiting an activity of GSK-3 or an active ingredient that is capable of downregulating an expression of GSK-3.

The biological condition according to the present invention is preferably is selected from the group consisting of obesity, non-insulin dependent diabetes mellitus, an insulin-dependent condition, an affective disorder, a neurodegenerative disease or disorder and a psychotic disease or disorder.

The affective disorder can be a unipolar disorder (e.g., depression) or a bipolar disorder (e.g., manic depression).

The neurodegenerative disorder can results from an event selected from the group consisting of cerebral ischemia, stroke, traumatic brain injury and bacterial infection, or can be a chronic neurodegenerative disorder that results from a disease selected from the group consisting of Alzheimer's disease, Huntington's disease, Parkinson's disease, AIDS associated dementia, amyotrophic lateral sclerosis (AML) and multiple sclerosis.

According to an additional aspect of the present invention there is provided a method of inhibiting an activity of GSK-3, which comprises contacting cells expressing GSK-3 with an inhibitory effective amount of a compound according to the present invention.

The activity can be a phosphorylation activity and/or an autophosphorylation activity.

According to yet an additional aspect of the present invention there is provided a method of potentiating insulin signaling, which comprises contacting insulin responsive cells with an effective amount of the compound described hereinabove.

In each of these methods, the contacting the cells can be effected *in vitro* or *in vivo*.

According to further features in preferred embodiments of the invention described below, each of the methods according to these additional aspects of the present invention further comprises contacting the cells with at least one an additional active ingredient, as is described hereinabove.

The present invention successfully addresses the shortcomings of the presently known configurations by providing newly designed, non-peptidic compounds for inhibiting GSK-3 activity, which can be efficiently used in the treatment of a variety of biological conditions.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the

patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

5 The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and
10 readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

15 In the drawings:

FIGs. 1a-b present computer images of the 3D structure of the peptides p9CREB (Figure 1a) and CREB (Figure 1b), as obtained by 2D ¹H-NMR studies (hydrogen atoms not shown; carbon backbone is in gray, nitrogen atoms are in blue, oxygen atoms are in red and phosphor atoms are in yellow);

20 FIG. 2 is an image showing the electrostatic distribution of the p9CREB peptide, based on the 3D structure of the peptide obtained by 2D ¹H-NMR studies;

FIG. 3 presents the chemical structures of phenyl phosphate, pyridoxal phosphate (P-5-P), GS-1, GS-2, GS-3 and of the novel compounds GS-4, GS-5 and GS-21;

25 FIGs. 4a-b present the ¹H NMR spectrum (Figure 4a) and the ¹³C NMR spectrum (Figure 4b) of 1,3,5-tris(hydroxymethyl)benzene, an intermediate in the synthesis of GS-21;

FIGs. 5a-b present the ¹H NMR spectrum (Figure 5a) and the ¹³C NMR spectrum (Figure 5b) of 3,5-Bis(bromomethyl)benzyl alcohol, an intermediate in the
30 synthesis of GS-21;

FIGs. 6a-b present the ¹H NMR spectrum (Figure 6a) and the ¹³C NMR spectrum (Figure 6b) of 3,5-Bis(cyanomethyl)benzyl alcohol, an intermediate in the synthesis of GS-21;

FIG. 7 presents the ^1H NMR spectrum of 3,5-bis(aminoethyl)benzyl alcohol, an intermediate in the synthesis of GS-21;

FIG. 8 presents the ^1H NMR spectrum of 3,5-bis(*tert*-butoxycarbonylaminoethyl)benzyl alcohol, an intermediate in the synthesis of GS-21;

5 FIGs. 9a-c present the ^1H NMR spectrum (Figure 9a) and the ^{13}C NMR spectrum (Figure 9b) and the ^{31}P NMR spectrum (Figure 9c) of protected 3,5-Bis(2-aminoethyl)benzyl phosphate, an intermediate in the synthesis of GS-21;

FIGs. 10a-d present the ^1H NMR spectrum (Figure 10a) and the ^{13}C NMR spectrum (Figure 10b), the ^{31}P NMR spectrum (Figure 10c) and the ESI-MS (Figure
10 10d) of the TFA salt 3,5-Bis(2-aminoethyl)benzyl phosphate (GS-21 TFA salt);

FIGs. 11a-e present the ^1H NMR spectrum (Figure 11a) and the ^{13}C NMR spectrum (Figure 11b), the ^{31}P NMR spectrum (Figure 11c), the ESI-MS (Figure 11d) and an HPLC chromatogram (Figure 11e) of 3,5-Bis(2-aminoethyl)benzyl phosphate (GS-21);

15 FIG. 12 presents comparative plots demonstrating the GSK-3 inhibition activity of phenyl phosphate, GS-1, GS-2, GS-3 and pyridoxal phosphate (P-5-P) in *in vitro* inhibition assays;

FIG. 13 presents comparative plots demonstrating the GSK-3 inhibition activity of GS-1, GS-2, GS-3, GS-5 and GS-21 in *in vitro* inhibition assays (Black
20 circles denote GS-1, red circles denote GS-2, green circles denote GS-3, blue circles denote GS-5 and pink circles denote GS-21); and

FIGs. 14a-b are bar graphs demonstrating the effect of GS-21 (Figure 14b) and GS-5 (Figure 14a) on glucose uptake in mouse adipocytes, represented by the $[\text{}^3\text{H}]$ 2-deoxy glucose incorporation in cells treated with GS-5 and GS-21 as fold activation
25 over cells treated with a peptide control (normalized to 1 unit).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of novel, non-peptidic compounds, which are capable of inhibiting GSK-3 activity and can therefore be used in the treatment of biological
30 conditions mediated by GSK-3. Specifically, the present invention is of (i) compounds that are designed according to the steric coordinates of a GSK-3 substrate, which may optionally have a hydrophobic moiety attached thereto; (ii) pharmaceutical compositions containing same; (iii) methods of using same for

inhibiting GSK-3 activity and potentiating insulin signaling; and (iv) methods of using same in the treatment of biological conditions such as, but not limited to, obesity, non-insulin dependent diabetes mellitus, insulin-dependent conditions, affective disorders, neurodegenerative diseases and disorders and psychotic diseases or disorders.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

One of the parameters that are responsible for substrate-kinase recognition is an element located within the substrate, which is usually related to as a "recognition motif". As is discussed hereinabove, GSK-3, unlike other kinases, has a unique recognition motif, which includes the amino acid sequence $SX_1X_2X_3S(p)$, set forth in SEQ ID NO:1, where S is serine or threonine, each of X_1 , X_2 and X_3 is any amino acid, and $S(p)$ is phosphorylated serine or phosphorylated threonine.

As is widely taught in WO 01/49709 and in U.S. Patent Application publication No. 20020147146, which are incorporated by reference as if fully set forth herein, a set of short peptides which were designed and synthesized based on this recognition motif were tested for their activity either as substrates or as inhibitors. Base on these assays, a number of features which rendered these peptides active substrates or inhibitors toward GSK-3, were determined. One of the most important features was that the phosphorylated serine or threonine residue in the motif is necessary for binding both substrates and inhibitors to GSK-3. These assays further demonstrated that some of these peptides were highly potent and specific inhibitors of GSK-3. These peptides were defined as substrate competitive inhibitors.

Based on the findings that GSK-3 recognizes only pre-phosphorylated substrates, namely, substrates that have a phosphorylated serine or threonine residue, it was hypothesized that these pre-phosphorylated GSK-3 substrates has a unique structure which allows them to interact with the catalytic core of GSK-3. It was

further hypothesized that determining this unique structure would enable the development of small molecules that could act as substrate competitive inhibitors of GSK-3.

Thus, in a search for small molecules that would mimic the inhibitory activity of the small GSK-3 peptide inhibitors described hereinabove, while reducing the present invention to practice, the three dimensional structure, as well as the unique structural features, of a short phosphorylated peptide substrate have been determined, and a number of compounds characterized by these features were tested for their activity as GSK-3 inhibitors.

As a representative example of a GSK-3 substrate the short pre-phosphorylated peptide p9CREB (ILSRRPS(p)YR, SEQ ID NO:2) was selected. The three-dimensional structures of p9CREB, as well as of the corresponding non-phosphorylated peptide CREB (ILSRRPSYR, SEQ ID NO:3) were determined by 2D NMR, as is detailed in the Examples section that follows.

As shown in Figures 1a and 1b, the phosphorylated p9CREB substrate has a defined structure in solution (Figure 1a), whereby the corresponding non-phosphorylated peptide CREB does not exhibit any unique structure (Figure 1b).

In view of these results it was suggested that the phosphate group in the phosphorylated peptide imposes a 'loop-like' structure, through a cation-pi interaction between tyrosine (Y8) and arginine (R4) (see, Tables 2 and 3), and as a result, the phosphorylated serine at the recognition motif is positioned outside the loop. Such a "bended" structure of the substrate renders the phosphorylated serine accessible to interact with the substrate binding pocket of the enzyme.

A support for this suggestion was indeed found in the recently published crystallization data of GSK-3, described by Dajani et al. (2001). The crystallization data of Dajani et al. show that the substrate binding site of GSK-3 comprises three positively charged residues, Arg 96, Arg 180, and Lys 205, which interact with a phosphate ion.

Figure 2 presents the electrostatic distribution on the 'surface' of the p9CREB peptide, based on these findings.

While continuing to conceive the present invention, it was deduced from the findings described hereinabove that a small molecule that would mimic the structure

15

of a GSK-3 substrate such that it would exerts substrate competitive inhibitory activity should be designed according to the following features:

The molecule should include a negatively charged group, preferably a phosphate group;

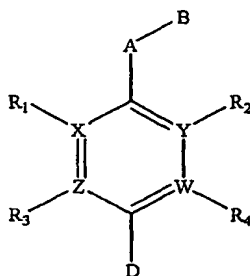
5 The negatively charged group should not be sterically hindered; and

The negatively charged group should preferably be flanked at least at one side thereof or at both sides by one or two positively charged groups.

Based on the above, a general formula of potential compounds for inhibiting GSK-3 activity has been designed. As is described in the Examples section that follows, preliminary experiments that were conducted with a 'first generation' of these compounds, namely, compounds having the most simplified structure of this formula, demonstrated the capability of these compounds to inhibit GSK-3 activity, thus providing a preliminary indication of the inhibitory potential of compounds having such a formula.

15 A more advanced generation of compounds, which includes novel compounds, was also designed and synthesized based on the above. As is described in the Examples section that follows, experiments conducted with these compounds further demonstrated their capability to inhibit GSK-3 activity and further to enhance the glucose uptake in mice adipocytes, thus demonstrating the promising inhibitory and therapeutic effect exerted by compounds designed according to such a formula

20 Hence, according to one aspect of the present invention there is provided a compound having a general formula:

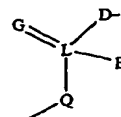


25

wherein:

X, Y, Z and W are each independently a carbon atom or a nitrogen atom;

A is alkyl or absent;



B is a negatively charged group having a formula $\text{G}=\text{L}(\text{D})(\text{E})\text{Q}^-$, wherein L is selected from the group consisting of a phosphor atom, a sulfur atom, a silicon atom, a boron atom and a carbon atom; Q, G and D are each independently selected from the group consisting of oxygen and sulfur;; and E is selected from the group consisting of hydroxy, alkoxy, aryloxy, carbonyl, thiocarbonyl, O-carboxy, thiohydroxy, thioalkoxy and thioaryloxy or absent;

D is selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, guanidinoalkyl, amino, aminoalkyl and a hydrophobic moiety; and

R_1 , R_2 , R_3 and R_4 are each independently selected from the group consisting of hydrogen, a lone pair of electrons, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanyliinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and an ammonium ion, or a pharmaceutically acceptable salt thereof.

It will be appreciated by one of skills in the art that the feasibility of each of the substituents (e.g., D, G, E, and R_1 - R_4) to be located at the indicated positions depends on the valency and chemical compatibility of the substituent, the substituted position and other substituents. Hence, the present invention is aimed at encompassing all the feasible substituents for any position.

As used herein, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; e.g., "1-20", is stated herein, it implies that the group, in this case the alkyl group, may contain 1 carbon atom, 2

carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. More preferably, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, unless otherwise indicated, the alkyl is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, ketoester, carbonyl, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and an ammonium ion.

A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and an ammonium ion.

An "alkenyl" group refers to an alkyl group, as defined hereinabove, which consists of at least two carbon atoms and at least one carbon-carbon double bond.

An "alkynyl" group refers to an alkyl group, as defined hereinabove, which consists of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are

phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and an ammonium ion.

A "heteroaryl" group refers to a monocyclic or fused ring (*i.e.*, rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide, ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and an ammonium ion.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. When substituted, the substituted group can be, for example, lone pair electrons, alkyl, hydroxyalkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonyl, sulfinyl, sulfonamide,

ketoester, carbonyl, thiocarbonyl, ester, ether, carboxy, thiocarboxy, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and an ammonium ion. Representative examples are piperidine, piperazine, tetrahydrofurane, tetrahydropyrane, morpholino and the like.

A "lone pair of electrons" refers to a pair of electrons that are not participating in a bond. The lone pair of electrons is present only when X, Y, Z or W is an unsubstituted nitrogen atom.

10 A "hydroxy" group refers to an -OH group.

An "azo" group refers to a -N=N group.

An "alkoxy" group refers to both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

15 An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "thiohydroxy" group refers to a -SH group.

A "thioalkoxy" group refers to both an -S-alkyl group, and an -S-cycloalkyl group, as defined herein.

20 A "thioaryloxy" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

A "carbonyl" group refers to a -C(=O)-R' group, where R' is hydrogen, alkyl, alkenyl, cycloalkyl, aryl, heteroaryl (bonded through a ring carbon) or heteroalicyclic (bonded through a ring carbon) as defined herein.

An "aldehyde" group refers to a carbonyl group, where R' is hydrogen.

25 A "thiocarbonyl" group refers to a -C(=S)-R' group, where R' is as defined herein for R'.

A "C-carboxy" group refers to a -C(=O)-O-R' groups, where R' is as defined herein.

30 An "O-carboxy" group refers to an R'C(=O)-O- group, where R' is as defined herein.

A "carboxylic acid" group refers to a C-carboxyl group in which R is hydrogen.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

A "trihalomethyl" group refers to a $-CX_3$ group wherein X is a halo group as defined herein.

A "trihalomethanesulfonyl" group refers to an $X_3CS(=O)_2-$ group wherein X is a halo group as defined herein.

5 A "sulfinyl" group refers to an $-S(=O)-R'$ group, where R' is as defined herein.

A "sulfonyl" group refers to an $-S(=O)_2-R'$ group, where R' is as defined herein.

10 An "S-sulfonamido" group refers to a $-S(=O)_2-NR'R''$ group, with R' as defined herein and R'' is as defined for R' .

An "N-sulfonamido" group refers to an $R'S(=O)_2-NR''$ group, where R' and R'' are as defined herein.

A "trihalomethanesulfonamido" group refers to an $X_3CS(=O)_2NR'-$ group, where R' and X are as defined herein.

15 An "O-carbamyl" group refers to an $-OC(=O)-NR'R''$ group, where R' and R'' are as defined herein.

A "N-carbamyl" group refers to an $R''OC(=O)-NR'-$ group, where R' and R'' are as defined herein.

20 An "O-thiocarbamyl" group refers to an $-OC(=S)-NR'R''$ group, where R' and R'' are as defined herein.

An "N-thiocarbamyl" group refers to an $R''OC(=S)NR'-$ group, where R' and R'' are as defined herein.

An "amino" group refers to an $-NR'R''$ group where R' and R'' are as defined herein.

25 An "aminoalkyl" group refers to an alkyl, as defined hereinabove, substituted by an amino group. Preferably, the alkyl terminates by the amino group.

A "C-amido" group refers to a $-C(=O)-NR'R''$ group, where R' and R'' are as defined herein.

30 An "N-amido" group refers to an $R'C(=O)-NR''$ group, where R' and R'' are as defined herein.

A "urea" group refers to an $-NR'C(=O)-NR''R'''$ group, where R' and R'' are as defined herein and R''' is defined as either R' or R'' .

A "guanidino" group refers to an $-R'NC(=NR''')-NR''R'''$ group, where R' , R'' and R''' are as defined herein and R'''' is defined as either R' , R'' or R''' .

A "guanidinoalkyl" group refers to an alkyl group substituted by a guanidino group, as these terms are defined herein. Preferably, the alkyl group terminates by the
5 guanidino group.

A "guanyl" group refers to an $R'R''NC(=NR''')-$ group, where R' , R'' , R''' and R'''' are as defined herein.

A "guanylinoalkyl" group refers to an alkyl group substituted by a guanyl group, as these terms are defined herein. Preferably, the alkyl group terminates by the
10 guanyl group.

A "nitro" group refers to a $-NO_2$ group.

A "cyano" group refers to a $-C\equiv N$ group.

The term "ketoester" describes a $-C(=O)-C(=O)-O-$ group.

The term "thiourea" describes a $-NR'-C(=S)-NR''-$ group, with R' and R'' as
15 defined hereinabove.

The term "hydrazine" describes a $NR'-NR''$ group, with R' and R'' as defined hereinabove.

The term "ammonium ion" described a $(NR'R''R''')^+$, where R' , R'' and R''' as defined hereinabove.

20 The compounds of the present invention are therefore based on a rigid structure, namely an aromatic (where X , Y , Z and W are all carbon atoms) or a heteroaromatic (where at least one of X , Y , Z and W is a nitrogen atom) ring, to which a negatively charged group is attached. As this structure mimics the unique structure of a GSK-3 substrate by providing a negatively charged group which is not
25 stearily hindered and has a geometrical structure similar or identical to a phosphate group, these compounds are capable of inhibiting GSK-3 activity.

The phrases "negatively charged group" and "positively charged group", as used herein, refer to an ionizable group, which upon ionization, typically in an aqueous medium, has at least one negative or positive electrostatic charge,
30 respectively. The charged groups can be present in the compounds of the present invention either in their ionized form or as a pre-ionized form.

The negatively charged group according to the present invention has a structure as defined hereinabove, whereby the positively charged group can be a

positively charged ion *per se* (e.g., an ammonium ion) or any group (e.g., alkyl, cycloalkyl, aryl, etc.) that is substituted by a positively charged ion (e.g., a secondary, tertiary or quaternary ammonium ion, an ionized aminoalkyl, etc.).

Preferably, the negatively charged group is a phosphate group, such that in the
5 formula above L is a phosphor atom, whereby each of Q, G and D is oxygen. Further preferably, E is hydroxy. Alternatively, the hydroxy group can also be ionized so as to have another negative electrostatic charge.

Alternatively, the negatively charged group can be a thiophosphate group, sulfate or sulfonate group, a borate or boronate group and the like, according to the
10 formula above.

The negatively charged group is preferably attached to the aryl/heteroaryl ring via an alkyl group, such that A in the formula above is alkyl, preferably an unsubstituted alkyl, and more preferably a methyl.

The attachment of the negatively charged group to the ring via an alkyl group
15 renders the negatively charged group a free rotatable group as opposed to its rigidity when attached directly to the ring. The free rotatability of the negatively charged group is advantageous since it enables the negatively charged group to readily interact with the binding site of the enzyme.

It should be noted herein that although the direct or indirect attachment of a
20 phosphate or any other negatively charged groups according to the present invention to an aromatic or heteroaromatic ring is effected via simple procedures and results in structurally simple compounds, only a limited number of such compounds have been synthesized hitherto. These include pyridoxal phosphate, benzyl phosphate, phenyl phosphate and a limited number of derivatives thereof (e.g., substituted pyridoxal
25 phosphate, benzyl phosphate and phenyl phosphate). It is assumed that since heretofore no biological activity has been associated with such compounds, one ordinarily skilled in the art was not motivated to provide such compounds. However, the compounds according to this aspect of the invention exclude any of the presently known compounds that are embraced by the formula above.

30 As is noted hereinabove, the base structure of the compounds of the present invention is an aromatic ring or a heteroaromatic ring.

However, since it is preferable to have one, and more preferably two, positively charged groups that flank the negatively charged group, the ring is

preferably a heteroaromatic ring, such that in the formula above at least one of X, Y, Z and W is a nitrogen atom. Preferably, Z or W is a nitrogen atom.

Further preferably at least two of X, Y, Z and W are nitrogen atoms, more preferably either X and Y are nitrogen atoms or Z and W are nitrogen atoms, and even
5 more preferably Z and W are nitrogen atoms.

As is well known in the art, a nitrogen atom within an aromatic ring is typically basic under neutral conditions and therefore, at a biological environment, it tends to be protonated so as to produce a positively charged $=NH^+$ group. As is described hereinabove, a compound that has one or two of such positively charged
10 groups flanking the negatively charged group is preferable.

As an alternative or in addition to the positively charged nitrogen atoms within the base ring, preferably at least one of R_1 , R_2 , R_3 and R_4 is a group containing at least one amino moiety.

As used herein, the phrase "group containing at least one amino moiety" refers
15 to those groups described above (e.g., alkyl, cycloalkyl, aryl, etc.) which contain one or more amino moiety, as this term is defined herein.

Representative examples of groups containing at least one amino moiety include, without limitation, an amino, an aminoalkyl, hydrazine, urea, thiourea, guanyl, amido, carbamyl, guanidino, guanidinoalkyl and guanyliinoalkyl, as these
20 terms are defined herein.

As is well known in the art, a free amino group is typically basic under neutral conditions and therefore, at a biological environment, it tends to be protonated so as to produce a positively charged $-NH_3^+$ group, for example. As is described hereinabove, a compound that has one or two of such positively charged groups flanking the
25 negatively charged group is preferable.

Thus, the amino moiety is preferably present in this group as a readily-protonated moiety, that is a moiety in which the amino nitrogen has a substantial partially negative charge.

Preferred examples of groups containing at least one amino moiety therefore
30 include, without limitation, an amino, an aminoalkyl, hydrazine, guanyl, guanyliinoalkyl, guanidino, guanidinoalkyl and guanyliinoalkyl, as these terms are defined herein.

The groups containing at least one amino moiety can be present in the compounds of the present invention either as is or as positively charged groups, in which at least one of the amino moieties is ionized.

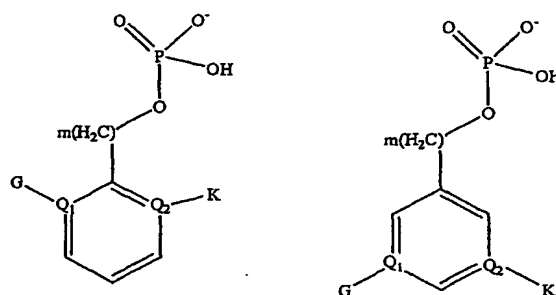
As is described above, positively charged groups according to the present invention comprise an ammonium ion, such that representative examples of positively charged groups include, without limitation, an ammonium ion *per se* (a protonated amino group) and any group that bears an ammonium ion, as is defined hereinabove, such as an alkyl, cycloalkyl or aryl substituted by an ammonium ion, guanidino, guanyl, hydrazine and the like.

Particularly preferred are positively charged groups that have a chemical structure derived from a side chain of a positively charged amino acid, e.g., lysine, arginine, histidine, proline and derivatives thereof, with the first two being the most preferred.

By "a chemical structure derived from a side chain of a positively charged amino acid" it is meant that the positively charged group has a similar or identical chemical structure as such a side chain.

Preferably either R_1 and R_2 or R_3 and R_4 are groups containing at least one amino moiety (e.g., positively charged groups), which flank the negatively charged group, as desired.

Hence, preferred compounds according to the present invention are those having the following formulas:



wherein m is an integer from 1 to 6; each of Q_1 and Q_2 is independently a carbon atom or a nitrogen atom; and G and/or K are each a group containing a free amino moiety (e.g., a positively charged group).

As is described above and is further demonstrated in the Examples section that follows, a number of compounds were designed according to the general formula described above, were successfully synthesized and were found to exert GSK-3 inhibition activity. The chemical structures of these compounds are presented in Figure 3. The efficacy of these compounds as inhibitors of GSK-3 activity is presented in Figures 12 and 13, whereby their beneficial effect on glucose uptake in mice adipocytes is demonstrated in Figures 14a and 14b.

As is shown in Figures 12 and 13 and in the Examples section that follows, some of the tested compounds do not have a positively charged group (e.g., GS-1 and GS-2, yet, these compounds exert inhibitory activity towards GSK-3. However, as is further shown in Figures 12 and 13, compounds that have a nitrogen atom within the base ring were found to be more active inhibitors, thus indicating a beneficial effect of such groups.

Hence, additional preferred compounds according to the present invention are compounds according to the general formula described above, in which each of X, Y, Z and W is a carbon atom; and at least one of R₃ and R₄ is a group containing an amino moiety (e.g., a positively charged group); and D is hydrogen or alkyl. More preferred compounds are those where each of X, Y and Z is a carbon atom and W is a nitrogen atom.

In another preferred embodiment of this aspect of the present invention, the compound has a hydrophobic moiety attached thereto.

As is described in detail in PCT/IL03/10157, by the present inventor, it was found that attaching a hydrophobic moiety to the N-terminus of GSK-3 peptide inhibitors enhances the inhibitory activity of the peptides.

Since the phosphorylated residue in the peptide inhibitors is located at the C-terminus thereof, it is assumed that compounds according to the present invention, which include a hydrophobic moiety that is located at the most distal position to the negatively charged group, as in the case of the peptide inhibitors, will exert enhanced inhibitory activity.

Hence, according to another aspect of the present invention there is provided a compound that has the general formula described hereinabove, where D is a hydrophobic moiety.

As used herein the phrase "hydrophobic moiety" refers to any substance or a residue thereof that is characterized by hydrophobicity.

As is well accepted in the art, the term "residue" describes a major portion of a substance that is covalently linked to another substance, herein the compound
5 described hereinabove.

Hence, a hydrophobic moiety according to the present invention is preferably a residue of a hydrophobic substance, and is preferably covalently attached to the compound described hereinabove.

Representative examples of hydrophobic substances from which the
10 hydrophobic moiety of the present invention can be derived include, without limitation, a saturated alkylene chain, an unsaturated alkylene chain, an aryl, a cycloalkyl and a hydrophobic peptide sequence.

As used herein, the phrase "alkylene chain" refers to a hydrocarbon linear chain, which can be saturated or unsaturated. The alkylene chain can be substituted or
15 unsubstituted, as is described above with respect to an alkyl group, and can be further interrupted by one or more heterogamous such as nitrogen, oxygen, sulfur, phosphor and the like. The alkylene chain preferably includes at least 4 carbon atoms, more preferably at least 8 carbon atoms, more preferably at least 10 carbon atoms and may have up to 20, 25 and even 30 carbon atoms.

20 The hydrophobic moiety of the present invention can therefore comprise a residue of the hydrophobic substances described hereinabove.

A preferred example of an alkylene chain according to this aspect of the present invention is an alkylene chain that comprises a carboxy group, namely, a fatty acid residue(s).

25 Preferred fatty acids that are usable in the context of the present invention include, without limitation, saturated or unsaturated fatty acids that have more than 10 carbon atoms, preferably between 12 and 24 carbon atoms, such as, but not limited to, myristic acid, lauric acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid etc.

30 Alternatively, the hydrophobic moiety, according to the present invention, can be a hydrophobic peptide sequence. The hydrophobic peptide sequence, according to the present invention, preferably includes between 2 and 15 amino acid residues, more preferably between 2 and 10 amino acid residues, more preferably between 2 and 5

amino acid residues, in which at least one amino acid residue is a hydrophobic amino acid residue.

Representative examples of hydrophobic amino acid residues include, without limitation, an alanine residue, a cysteine residue, a glycine residue, an isoleucine residue, a leucine residue, a valine residue, a phenylalanine residue, a tyrosine residue, a methionine residue, a proline residue and a tryptophan residue, or any modification thereof, as is described hereinabove.

Alternatively, the hydrophobic amino acid residue can include any other amino acid residue, which has been modified by incorporation of a hydrophobic moiety thereto.

As used herein, the phrase "amino acid residue", which is also referred to herein, interchangeably, as "amino acid", describes an amino acid unit within a polypeptide chain. The amino acid residues within the hydrophobic peptide sequence can be either natural or modified amino acid residues, as these phrases are defined hereinafter.

As used herein, the phrase "natural amino acid residue" describes an amino acid residue, as this term is defined hereinabove, which includes one of the twenty amino acids found in nature.

As used herein, the phrase "modified amino acid residue" describes an amino acid residue, as this term is defined hereinabove, which includes a natural amino acid that was subjected to a modification at its side chain. Such modifications are well known in the art and include, for example, incorporation of a functionality group such as, but not limited to, a hydroxy group, an amino group, a carboxy group and a phosphate group within the side chain. This phrase therefore includes, unless otherwise specifically indicated, chemically modified amino acids, including amino acid analogs (such as penicillamine, 3-mercapto-D-valine), naturally-occurring non-proteogenic amino acids (such as norleucine), and chemically-synthesized compounds that have properties known in the art to be characteristic of an amino acid. The term "proteogenic" indicates that the amino acid can be incorporated into a protein in a cell through well-known metabolic pathways.

Accordingly, as used herein, the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including, for example, hydroxyproline,

phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids which are linked via a peptide bond or a peptide bond analog to at least one addition amino acid as this term is defined herein.

As the hydrophobic moiety provides for enhanced unpredictable activity, known compounds such as phenyl phosphate and pyridoxal phosphate, which are substituted by a hydrophobic moiety, are also included within the scope of this aspect of the present invention.

As is discussed hereinabove and is further demonstrated in the Examples section that follows, the compounds of the present invention, described hereinabove, are designed based on the three-dimensional structure of a GSK-3 substrate and are therefore potential substrate competitive inhibitors of GSK-3 activity.

Hence, according to still another aspect of the present invention, there is provided a method of inhibiting an activity of GSK-3, which is effected by contacting cells expressing GSK-3 with an inhibitory effective amount of a compound described hereinabove.

As used herein, the term "inhibitory effective amount" is the amount determined by such considerations as are known in the art, which is sufficient to inhibit the activity of GSK-3. The activity can be a phosphorylation and/or autophosphorylation activity of GSK-3.

The method according to this aspect of the present invention can be effected by contacting the cells with the compounds *in vitro* and/or *in vivo*. This method can be further effected by further contacting the cells with an additional active ingredient that is capable of altering an activity of GSK-3, as is detailed hereinbelow.

The inhibition of GSK-3 activity is a way to increase insulin activity *in vivo*. High activity of GSK-3 impairs insulin action in intact cells (Eldar-Finkelman et al, 1997). This impairment results from the phosphorylation of insulin receptor substrate-1 (IRS-1) serine residues by GSK-3. Studies performed in patients with type II diabetes (non-insulin dependent diabetes mellitus, NIDDM) show that glycogen synthase activity is markedly decreased in these patients, and that decreased activation of protein kinase B (PKB), an upstream regulator of GSK-3, by insulin is also detected (Shulman et al, (1990); Nikoulina et al, (1997); Cross et al, (1995).

Mice susceptible to high fat diet-induced diabetes and obesity have significantly increased GSK-3 activity in epididymal fat tissue (Eldar-Finkelman et al, 1999). Increased GSK-3 activity expressed in cells resulted in suppression of glycogen synthase activity (Eldar-Finkelman et al, 1996).

5 Inhibition of GSK-3 activity therefore provides a useful method for increasing insulin activity in insulin-dependent conditions. Thus, according to yet another aspect of the present invention there is provided a method of potentiating insulin signaling, which is effected by contacting insulin responsive cells with an effective amount, as is defined hereinabove, of a compound according to the present invention.

10 As used herein, the phrase "potentiating insulin signaling" includes an increase in the phosphorylation of insulin receptor downstream components and an increase in the rate of glucose uptake as compared with glucose uptake in untreated subjects or cells.

 The method according to this aspect of the present invention can be effected
15 by contacting the cells with the compound of the present invention, *in vitro* or *in vivo*, and can be also effected by further contacting the cells with insulin.

 Potentiation of insulin signaling, *in vivo*, resulting from administration of the conjugates of the present invention, can be monitored as a clinical endpoint. In principle, the easiest way to look at insulin potentiation in a patient is to perform the
20 glucose tolerance test. After fasting, glucose is given to a patient and the rate of the disappearance of glucose from blood circulation (namely glucose uptake by cells) is measured by assays well known in the art. Slow rate (as compared to healthy subject) of glucose clearance will indicate insulin resistance. The administration of an
25 inhibitor to an insulin-resistant patient increases the rate of glucose uptake as compared with a non-treated patient. The inhibitor may be administered to an insulin resistant patient for a longer period of time, and the levels of insulin, glucose, and leptin in blood circulation (which are usually high) may be determined. Decrease in
glucose levels will indicate that the inhibitor potentiated insulin action. A decrease in
insulin and leptin levels alone may not necessarily indicate potentiation of insulin
30 action, but rather will indicate improvement of the disease condition by other mechanisms.

 The compounds of the present invention, described hereinabove, can be effectively utilized for treating any biological condition that is associated with GSK-3.

Hence, according to an additional aspect of the present invention, there is provided a method of treating a biological condition associated with GSK-3 activity. The method, according to this aspect of the present invention, is effected by administering to a subject in need thereof a therapeutically effective amount of the compound of the present invention, described hereinabove.

The phrase "biological condition associated with GSK-3 activity" as used herein includes any biological or medical condition or disorder in which effective GSK-3 activity is identified, whether at normal or abnormal levels. The condition or disorder may be caused by the GSK-3 activity or may simply be characterized by GSK-3 activity. That the condition is associated with GSK-3 activity means that some aspect of the condition can be traced to the GSK-3 activity.

Herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition or disorder, substantially ameliorating clinical symptoms of a condition or disorder or substantially preventing the appearance of clinical symptoms of a condition or disorder. These effects may be manifested, for example, by a decrease in the rate of glucose uptake with respect to type II diabetes or by halting neuronal cell death with respect to neurodegenerative disorders, as is detailed hereinbelow.

The term "administering" as used herein describes a method for bringing the compound of the present invention and cells affected by the condition or disorder together in such a manner that the compound can affect the GSK-3 activity in these cells. The compounds of the present invention can be administered via any route that is medically acceptable. The route of administration can depend on the disease, condition or injury being treated. Possible administration routes include injections, by parenteral routes, such as intravascular, intravenous, intra-arterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular, intraepidural, intracerebrovascular or others, as well as oral, nasal, ophthalmic, rectal, topical, or by inhalation. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants. Administration can also be intra-articularly, intrarectally, intraperitoneally, intramuscularly, subcutaneously, or by aerosol inhalant. Where treatment is systemic, the compound can be administered orally or parenterally, such as intravenously, intramuscularly, subcutaneously, intraorbitally, intracapsularly, intraperitoneally or intracisternally, as

long as provided in a composition suitable for effecting the introduction of the compound into target cells, as is detailed hereinbelow.

The phrase "therapeutically effective amount", as used herein, describes an amount administered to an individual, which is sufficient to abrogate, substantially inhibit, slow or reverse the progression of a condition associated with GSK-3 activity, to substantially ameliorate clinical symptoms of a such a condition or substantially prevent the appearance of clinical symptoms of such a condition. The GSK-3 activity can be a GSK-3 kinase activity. The inhibitory amount may be determined directly by measuring the inhibition of a GSK-3 activity, or, for example, where the desired effect is an effect on an activity downstream of GSK-3 activity in a pathway that includes GSK-3, the inhibition may be measured by measuring a downstream effect. Thus, for example where inhibition of GSK-3 results in the arrest of phosphorylation of glycogen synthase, the effects of the compound may include effects on an insulin-dependent or insulin-related pathway, and the compound may be administered to the point where glucose uptake is increased to optimal levels. Also, where the inhibition of GSK-3 results in the absence of phosphorylation of a protein that is required for further biological activity, for example, the tau protein, then the compound may be administered until polymerization of phosphorylated tau protein is substantially arrested. Therefore, the inhibition of GSK-3 activity will depend in part on the nature of the inhibited pathway or process that involves GSK-3 activity, and on the effects that inhibition of GSK-3 activity has in a given biological context.

The amount of the compound that will constitute an inhibitory amount will vary depending on such parameters as the compound and its potency, the half-life of the compound in the body, the rate of progression of the disease or biological condition being treated, the responsiveness of the condition to the dose of treatment or pattern of administration, the formulation, the attending physician's assessment of the medical situation, and other relevant factors, and in general the health of the patient, and other considerations such as prior administration of other therapeutics, or co-administration of any therapeutic that will have an effect on the inhibitory activity of the compound or that will have an effect on GSK-3 activity, or a pathway mediated by GSK-3 activity. It is expected that the inhibitory amount will fall in a relatively broad range that can be determined through routine trials.

As is discussed in detail hereinabove, GSK-3 is involved in various biological pathways and hence, the method according to this aspect of the present invention can be used in the treatment of a variety of biological conditions, as is detailed hereinunder.

5 GSK-3 is involved in the insulin signaling pathway and therefore, in one example, the method according this aspect of the present invention can be used to treat any insulin-dependent condition.

As GSK-3 inhibitors are known to inhibit differentiation of pre-adipocytes into adipocytes, in another example, the method of this aspect of the present invention can
10 be used to treat obesity.

In yet another example, the method according to this aspect of the present invention can be used to treat diabetes and particularly, non-insulin dependent diabetes mellitus.

Diabetes mellitus is a heterogeneous primary disorder of carbohydrate
15 metabolism with multiple etiologic factors that generally involve insulin deficiency or insulin resistance or both. Type I, juvenile onset, insulin-dependent diabetes mellitus, is present in patients with little or no endogenous insulin secretory capacity. These patients develop extreme hyperglycemia and are entirely dependent on exogenous insulin therapy for immediate survival. Type II, or adult onset, or non-insulin-
20 dependent diabetes mellitus, occurs in patients who retain some endogenous insulin secretory capacity, but the great majority of them are both insulin deficient and insulin resistant. Approximately 95 % of all diabetic patients in the United States have non-insulin dependent, Type II diabetes mellitus (NIDDM), and, therefore, this is the form of diabetes that accounts for the great majority of medical problems. Insulin
25 resistance is an underlying characteristic feature of NIDDM and this metabolic defect leads to the diabetic syndrome. Insulin resistance can be due to insufficient insulin receptor expression, reduced insulin-binding affinity, or any abnormality at any step along the insulin signaling pathway (see U.S. Patent No. 5,861,266).

The compounds of the present invention can be used to treat type II diabetes in
30 a patient with type II diabetes as follows: a therapeutically effective amount of the compound is administered to the patient, and clinical markers, e.g., blood sugar level, are monitored. The compounds of the present invention can further be used to prevent type II diabetes in a subject as follows: a prophylactically effective amount of

the compound is administered to the patient, and a clinical marker, for example IRS-1 phosphorylation, is monitored.

Treatment of diabetes is determined by standard medical methods. A goal of diabetes treatment is to bring sugar levels down to as close to normal as is safely possible. Commonly set goals are 80–120 milligrams per deciliter (mg/dl) before meals and 100–140 mg/dl at bedtime. A particular physician may set different targets for the patient, depending on other factors, such as how often the patient has low blood sugar reactions. Useful medical tests include tests on the patient's blood and urine to determine blood sugar level, tests for glycated hemoglobin level (HbA_{1c}; a measure of average blood glucose levels over the past 2–3 months, normal range being 4–6 %), tests for cholesterol and fat levels, and tests for urine protein level. Such tests are standard tests known to those of skill in the art (see, for example, American Diabetes Association, 1998). A successful treatment program can also be determined by having fewer patients in the program with diabetic eye disease, kidney disease, or nerve disease.

Hence, in one particular embodiment of the method according to this aspect of the present invention, there is provided a method of treating non-insulin dependent diabetes mellitus: a patient is diagnosed in the early stages of non-insulin dependent diabetes mellitus. A compound of the present invention is formulated in an enteric capsule. The patient is directed to take one tablet after each meal for the purpose of stimulating the insulin signaling pathway, and thereby controlling glucose metabolism to levels that obviate the need for administration of exogenous insulin

As is further discussed hereinabove, and has been demonstrated in the PCT International Patent Application entitled "Glycogen Synthase Kinase-3 Inhibitors", by the same applicant, which is filed on the same date as the instant application, GSK-3 inhibition is associated with affective disorders. Therefore, in another example, the method according to this aspect of the present invention can be used to treat affective disorders such as unipolar disorders (e.g., depression) and bipolar disorders (e.g., manic depression).

As GSK-3 is also considered to be an important player in the pathogenesis of neurodegenerative disorders and diseases, the method according to this aspect of the present invention can be further used to treat a variety of such disorders and diseases.

In one example, since inhibition of GSK-3 results in halting neuronal cell death, the method according to this aspect of the present invention can be used to treat a neurodegenerative disorder that results from an event that cause neuronal cell death. Such an event can be, for example, cerebral ischemia, stroke, traumatic brain injury or
5 bacterial infection.

In another example, since GSK-3 activity is implicated in various central nervous system disorders and neurodegenerative diseases, the method according to this aspect of the present invention can be used to treat various chronic neurodegenerative diseases such as, but not limited to, Alzheimer's disease,
10 Huntington's disease, Parkinson's disease, AIDS associated dementia, amyotrophic lateral sclerosis (AML) and multiple sclerosis.

As is discussed hereinabove, GSK-3 activity has particularly been implicated in the pathogenesis of Alzheimer's disease. Hence, in one representative embodiment of the method according to this aspect of the present invention, there is provided a
15 method of treating a patient with Alzheimer's disease: A patient diagnosed with Alzheimer's disease is administered with a compound of the present invention, which inhibits GSK-3-mediated tau hyperphosphorylation, prepared in a formulation that crosses the blood brain barrier (BBB). The patient is monitored for tau phosphorylated polymers by periodic analysis of proteins isolated from the patient's
20 brain cells for the presence of phosphorylated forms of tau on an SDS-PAGE gel known to characterize the presence of and progression of the disease. The dosage of the compound is adjusted as necessary to reduce the presence of the phosphorylated forms of tau protein.

GSK-3 has also been implicated with respect to psychotic disorders such as
25 schizophrenia, and therefore the method according to this aspect of the present invention can be further used to treat psychotic diseases or disorders, such as schizophrenia.

The method according to this aspect of the present invention can be further effected by co-administering to the subject one or more additional active ingredient(s)
30 which is capable of altering an activity of GSK-3.

As used herein, "co-administering" describes administration of a compound according to the present invention in combination with the additional active ingredient(s) (also referred to herein as active or therapeutic agent). The additional

active agent can be any therapeutic agent useful for treatment of the patient's condition. The co-administration may be simultaneous, for example, by administering a mixture of the compound and the therapeutic agents, or may be accomplished by administration of the compound and the active agents separately, such as within a short time period. Co-administration also includes successive administration of the compound and one or more of another therapeutic agent. The additional therapeutic agent or agents may be administered before or after the compound. Dosage treatment may be a single dose schedule or a multiple dose schedule.

10 The additional active ingredient can be insulin.

Preferably, the additional active ingredient is capable of inhibiting an activity of GSK-3, such that the additional active ingredient according to the present invention can be any GSK-3 inhibitor other than the compounds of the present invention, e.g., a short peptide GSK-3 inhibitor as described in WO 01/49709, PCT/IL03/01057 and 15 U.S. Patent Application Publication No. 20020147146A1. Alternatively, the GSK-3 inhibitor can be, for example, lithium, valproic acid and/or lithium ion.

Alternatively, the additional active ingredient can be an active ingredient that is capable of downregulating an expression of GSK-3.

An agent that downregulates GSK-3 expression refers to any agent which 20 affects GSK-3 synthesis (decelerates) or degradation (accelerates) either at the level of the mRNA or at the level of the protein. For example, a small interfering polynucleotide molecule which is designed to down regulate the expression of GSK-3 can be used as an additional active ingredient according to this embodiment of the present invention.

25 An example for a small interfering polynucleotide molecule which can down-regulate the expression of GSK-3 is a small interfering RNA or siRNA, such as, for example, the morpholino antisense oligonucleotides described by in Munshi et al. (Munshi CB, Graeff R, Lee HC, *J Biol Chem* 2002 Dec 20;277(51):49453-8), which includes duplex oligonucleotides which direct sequence specific degradation of 30 mRNA through the previously described mechanism of RNA interference (RNAi) (Hutvagner and Zamore (2002) *Curr. Opin. Genetics and Development* 12:225-232).

As used herein, the phrase "duplex oligonucleotide" refers to an oligonucleotide structure or mimetics thereof, which is formed by either a single self-

complementary nucleic acid strand or by at least two complementary nucleic acid strands. The "duplex oligonucleotide" of the present invention can be composed of double-stranded RNA (dsRNA), a DNA-RNA hybrid, single-stranded RNA (ssRNA), isolated RNA (i.e., partially purified RNA, essentially pure RNA), synthetic RNA and
5 recombinantly produced RNA.

Preferably, the specific small interfering duplex oligonucleotide of the present invention is an oligoribonucleotide composed mainly of ribonucleic acids.

Instructions for generation of duplex oligonucleotides capable of mediating RNA interference are provided in www.ambion.com.

10 Hence, the small interfering polynucleotide molecule according to the present invention can be an RNAi molecule (RNA interference molecule).

Alternatively, a small interfering polynucleotide molecule can be an oligonucleotide such as a GSK-3-specific antisense molecule or a ribozyme molecule, further described hereinunder.

15 Antisense molecules are oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target
20 polynucleotide. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. An example for such includes RNase H, which is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide
25 inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

30 The antisense molecules of the present invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, as described above. Representative U.S. patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797;

5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein fully incorporated by reference.

Rybozyme molecules are being increasingly used for the sequence-specific inhibition of gene expression by the cleavage of mRNAs. Several rybozyme sequences can be fused to the oligonucleotides of the present invention. These sequences include but are not limited to ANGIOZYME specifically inhibiting formation of the VEGF-R (Vascular Endothelial Growth Factor receptor), a key component in the angiogenesis pathway, and HEPTAZYME, a rybozyme designed to selectively destroy Hepatitis C Virus (HCV) RNA, (Rybozyme Pharmaceuticals, Incorporated - WEB home page).

Further alternatively, a small interfering polynucleotide molecule, according to the present invention can be a DNAzyme.

DNAzymes are single-stranded catalytic nucleic acid molecules. A general model (the "10-23" model) for the DNAzyme has been proposed. "10-23" DNAzymes have a catalytic domain of 15 deoxyribonucleotides, flanked by two substrate-recognition domains of seven to nine deoxyribonucleotides each. This type of DNAzyme can effectively cleave its substrate RNA at purine:pyrimidine junctions (Santoro, S.W. & Joyce, G.F. Proc. Natl. Acad. Sci. USA 199; for rev of DNAzymes see Khachigian, LM Curr Opin Mol Ther 2002;4:119-21).

Examples of construction and amplification of synthetic, engineered DNAzymes recognizing single and double-stranded target cleavage sites have been disclosed in U.S. Pat. No. 6,326,174 to Joyce et al. DNAzymes of similar design directed against the human Urokinase receptor were recently observed to inhibit Urokinase receptor expression, and successfully inhibit colon cancer cell metastasis in vivo (Itoh et al., 20002, Abstract 409, Ann Meeting Am Soc Gen Ther www.asgt.org). In another application, DNAzymes complementary to bcr-abl oncogenes were successful in inhibiting the oncogenes expression in leukemia cells, and lessening relapse rates in autologous bone marrow transplant in cases of CML and ALL.

Oligonucleotides designed according to the teachings of the present invention can be generated according to any oligonucleotide synthesis method known in the art such as enzymatic synthesis or solid phase synthesis. Equipment and reagents for

executing solid-phase synthesis are commercially available from, for example, Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the capabilities of one skilled in the art.

5 While being potent therapeutic agents, and since therapeutic applications often require administration of effective amounts of an active ingredient to a treated individual, the compounds of the present invention are preferably included, as active ingredients, in a pharmaceutical composition which further comprises a pharmaceutically acceptable carrier for facilitating administration of a compound to
10 the treated individual and possibly to facilitate entry of the active ingredient into the targeted tissues or cells.

Hence, according to still an additional aspect of the present invention there is provided a pharmaceutical composition which comprises, as an active ingredient, a compound according to the present invention and a pharmaceutically acceptable
15 carrier.

Hereinafter, the phrases "pharmaceutically acceptable carrier" and "physiologically acceptable carrier" refer to a carrier or a diluent that does not cause significant irritation to a subject and does not abrogate the biological activity and properties of the administered compound. Examples, without limitations, of carriers
20 are propylene glycol, saline, emulsions and mixtures of organic solvents with water.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable
25 oils and polyethylene glycols.

The pharmaceutical acceptable carrier can further include other agents such as, but not limited to, absorption delaying agents, antibacterial agents, antifungal agents, antioxidant agents, binding agents, buffering agents, bulking agents, cationic lipid agents, coloring agents, diluents, disintegrants, dispersion agents, emulsifying agents, excipients, flavoring agents, glidants, isotonic agents, liposomes, microcapsules,
30 solvents, sweetening agents, viscosity modifying agents, wetting agents, and skin penetration enhancers.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, 5 transmucosal, transdermal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, 10 granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more pharmaceutically acceptable carriers comprising excipients and auxiliaries, which facilitate processing 15 of the compound into preparations which can be used pharmaceutically. The composition can be formulated in a delivery form such as an aerosol delivery form, aqueous solution, bolus, capsule, colloid, delayed release, depot, dissolvable powder, drops, emulsion, erodible implant, gel, gel capsule, granules, injectable solution, ingestible solution, inhalable solution, lotion, oil solution, pill, suppository, salve, 20 suspension, sustained release, syrup, tablet, tincture, topical cream, transdermal delivery form. Proper formulation is dependent upon the route of administration chosen.

For injection, the compound of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, 25 Ringer's solution, or physiological saline buffer with or without organic solvents such as propylene glycol, polyethylene glycol. For transmucosal administration, penetrants are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compound can be formulated readily by combining the compound with pharmaceutically acceptable carriers well known in the 30 art. Such carriers enable the compound of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the

mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl
5 cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose,
10 concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active ingredient doses.

15 Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft
20 capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or
25 lozenges formulated in conventional manner.

For administration by inhalation, the compound according to the present invention is conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or
30 carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated

containing a powder mix of the ingredient and a suitable powder base such as lactose or starch.

The compound described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose
5 containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous
10 solutions of the compound in water-soluble form. Additionally, suspensions of the compound may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension,
15 such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredient to allow for the preparation of highly concentrated solutions.

Alternatively, the compound may be in powder form for constitution with a
20 suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compound of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

The pharmaceutical compositions herein described may also comprise suitable
25 solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin and polymers such as polyethylene glycols.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the compound is contained in an amount
30 effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of a compound effective to affect symptoms of a condition or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any active ingredient used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from activity
5 assays in cell cultures and/or animals. Such information can be used to more accurately determine useful doses in humans.

The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage
10 can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as a FDA approved kit, which may contain one or more unit dosage forms containing the compound. The pack may, for example, comprise metal
15 or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions
20 or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable
25 conditions indicated on the label may include, for example, any of the biological conditions associated with GSK-3 activity listed hereinabove.

Hence, the pharmaceutical composition of the present invention can be packaged in a packaging material and identified in print, on or in the packaging material, for use in the treatment or prevention of a biological condition associated with GSK-3.

The pharmaceutical composition of the present invention can further comprises an additional active ingredient that is capable of interfering with an activity of GSK-3, as is described hereinabove.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

MATERIALS AND EXPERIMENTAL METHODS

Materials:

Peptides were synthesized by Genemed Synthesis Inc. (San Francisco, CA).

Radioactive materials were purchased from Amersham Ltd.

Phenyl phosphate and pyridoxal phosphate (also referred to herein as P-5-P) were obtained from Sigma (Israel).

All reagents and solvents were obtained from commercial sources (e.g., Sigma, Acros, Aldrich) and were used as supplied, unless otherwise indicated.

GS-1, GS-2 and GS-3 were synthesized according to procedures known in the art, as is detailed hereinunder.

Syntheses of the novel compounds GS-4, GS-5 and GS-21 were designed and practiced as described hereinbelow.

Determination of a 3D structure of a GSK-3 substrate by NMR Spectroscopy and Structure Calculations:

A small phosphorylated peptide patterned after the known GSK-3 substrate CREB, denoted p9CREB, and two additional peptides, a non-phosphorylated peptide, 9CREB, and a variant where S¹ was replaced with glutamic acid (which is thought to mimic a charged group), 9ECREB, were used in these studies and are listed in Table 1 below. Time course analyses of peptide phosphorylation by GSK-3 confirmed that only the phosphorylated peptide, p9CREB, was a substrate for GSK-3, while 9CREB and 9ECREB completely failed to be phosphorylated by GSK-3 (data not shown),

thus indicating again that phosphorylated serine is an absolute requirement for GSK-3.

Table 1

Peptide	Sequence	SEQ ID NO:
p9CREB	IL <u>S</u> RRPS(p)YR	2
9CREB	IL <u>S</u> RRPSYR	3
9ECREB	IL <u>S</u> RRPEYR	4

5 The 3D structure of p9CREB (Figure 1a), 9CREB (Figure 1b) and 9ECREB (not shown) by 2D ¹H NMR spectroscopy was determined using the following procedures:

For the structural studies, a solution of each peptide was prepared by dissolving lyophilized powder in water containing 10 % D₂O. 2D-NMR spectra were
10 acquired at the ¹H proton frequency of 600.13 MHz on a Bruker Avance DMX spectrometer. The carrier frequency was set on the water signal and it was suppressed by applying either a WATERGATE method and by low-power irradiation during the relaxation period. The experimental temperature (280 K) was optimized in order to reduce population averaging due to the fast exchange at more ambient temperatures,
15 while preserving the best possible spectral resolution. All experiments were carried out in the phase sensitive mode (TPPI or States-TPPI) and recorded with a spectral width of 12 ppm, with 4K real t₂ data points and 512 t₁-increments. Two-dimensional homonuclear data collected included TOCSY using a MLEV pulse sequence with a mixing time of 150 msec, and NOESY experiments with mixing times ranging
20 between 100 and 750 msec. Typically, the relaxation delays were 1.5 and 2.0 sec in TOCSY and NOESY experiments, respectively. In the ROESY measurements, the duration of the spin-lock was set to 400 msec with a power of 3.4 KHz. All spectra were calibrated versus tetramethylsilane.

The data was processed using Bruker XWINNMR software (Bruker
25 Analytische Messtechnik, GmbH, version 2.7). All data processing, calculations and analysis were done on Silicon Graphics workstations (INDY R4000 and INDIGO2 R10000). Zero filling of the indirect dimension and apodization of the free induction decay by a shifted squared-sine window function on both dimensions were applied prior to Fourier transformation to enhance spectral resolution. The spectra were

further phase-corrected by applying an automatic polynomial baseline correction developed by Bruker.

Resonance assignment was based on the TOCSY and NOESY spectra measured at the same experimental conditions, according to the sequential assignment methodology developed by Wüthrich using the Bruker software program AURELIA (Bruker Analytic GmbH, version 2.7).

The NOE distance restraints were derived from NOESY spectra recorded at 450 msec. This optimal mixing time was determined for the p9CREB peptide sample by comparing the NOE signal intensities in a series of experiments with mixing times varying from 100 msec to 750 msec. The chosen mixing time gave maximal NOE buildup with no significant contribution from spin diffusion. This value was used for the non-phosphorylated analog experiment in order to maintain identical experimental conditions. Integrated peak volumes were converted into distance restraints using a r^{-6} dependency and the known distance of 2.47 Å between the two adjacent protons of the tyrosine aromatic ring was used for calibration. The restraints were classified into strong (1.8-2.5 Å), medium (1.8-3.5 Å) and weak (1.8-5.0 Å). An empirical correction of 0.5 Å was added to the upper bound for restraints involving methyl groups.

The structures were calculated by hybrid distance geometry – dynamical simulated annealing using XPLOR (version 3.856). The NOE energy was introduced as a square-well potential with a constant force constant of 50 Kcal/mol·Å². Simulated annealing consisted of 1500 3 fsec steps at 1000 K and 3000 1fsec steps during cooling to 300 K. Finally, the structures were minimized using conjugate gradient energy minimization for 4000 iterations. INSIGHTII (Molecular Modeling System version 97.0, Molecular Simulations, Inc.) was used for visualization and analysis of the NMR-derived structures. Their quality was assessed using PROCHECK.

Analytical methods:

Proton, carbon, fluorine and phosphorus nuclear magnetic resonance spectra were obtained on either a Bruker AMX 500 spectrometer or a Bruker AV 300 spectrometer and are reported in ppm (δ). Tetramethylsilane (TMS) was used as an internal standard for proton spectra, phosphoric acid was used as an internal standard

for phosphorus spectra and the solvent peak was used as the reference peak for carbon and fluorine spectra.

Mass spectra were obtained on a Finnigan LCQ Duo LC-MS ion trap electrospray ionization (ESI) mass spectrometer.

5 Thin-layer chromatography (TLC) was performed using Analtech silica gel plates and visualized by ultraviolet (UV) light, or by staining the plates in 0.2 wt % ninhydrine in butanol.

Elemental analysis was performed by Quantitative Technologies, Inc. (Whitehouse, NJ).

10 HPLC analyses were obtained using a Hypersil BDS C18 Column, 4.6 × 150 mm, 5 μm, Column Temperature Ambient, Detector @ 220 nm using a standard solvent gradient program, as follows:

Time (Minutes)	Flow (mL/min)	%A	%B
0.0	1.0	100	0.0
4.0	1.0	100	0.0
20.0	1.0	92.0	8.0
21.0	1.0	100	0.0
22.0	1.0	100	0.0

A = 0.1 % TFA in water

15 B = 0.1 % TFA in acetonitrile

In vitro inhibition assays:

Purified recombinant rabbit GSK-3β (Eldar-Finkelman et al., 1996) was incubated with peptide substrate PGS-1 (YRRAAVPPSPSLSRHSSPSQS(p)EDEEE) (SEQ ID NO:1) and with phenyl phosphate, pyridoxal phosphate (P-5-P), GS-1, GS-2, GS-3, GS-5 or GS-21 (structural formulas are depicted in Figure 3), at indicated concentrations. The reaction mixture included Tris 50 mM (pH = 7.3), 10 mM MgAc, ³²P[γ-ATP] (100 μM), 0.01 % β-mercaptoethanol, and was incubated for 10 minutes at 30 °C. Reactions were spotted on phosphocellulose paper (p81), washed with 100 mM phosphoric acid, and counted for radioactivity (as described in Eldar-Finkelman et al., 1996).

25 *Glucose uptake in isolated adipocytes:* Mice adipocytes were isolated from epididymal fat pad by digestion with 0.8 mg/ml collagenase (Worthington Biochemical) as described previously (Lawrence et al., 1977). Digested fat pads were passed through nylon mesh and cells were washed 3 times with Krebs-bicarbonate buffer (pH = 7.4) containing 1 % bovine serum albumin (Fraction V,

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Boehringer Mannheim, Germany), 10 mM HEPES (pH = 7.3), 5 mM glucose and 200 nM adenosine. Cells were incubated with GS-5 and GS-21 at indicated concentrations for 2.5 hours, followed by addition of 2-deoxy [³H] glucose (0.5 μ ci/vial) for 10 minutes. The assay was terminated by centrifugation of cells through
5 dinonylphthalate (ICN, USA). ³H was thereafter quantitated by liquid scintillation analyzer (Packard). Nonspecific uptake of 2-deoxy-[³H] glucose was determined by the addition of cytochalasin B (50 μ M) 30 minutes prior to the addition of radioactive material.

10

EXPERIMENTAL RESULTS

Determination of a 3D structure of a GSK-3 substrate:

Tables 2 and 3 below present the structural coordinate data that was used for inputting into structure analysis software for visualization of the 3D structures.

15 The obtained 3D structures, presented in Figures 1a and 1b, it was observed that only the phosphorylated peptide has a defined structural conformation. As is shown in Figure 1a, for p9CREB, the phosphorylation imposed a significant "turn" of the peptide backbone, bringing Tyr 8 and Arg 4 closer, and forming a 'loop structure' whereby the phosphorylated residue is pointing out of the loop. This conformation
20 minimizes on the one hand interference of positively charged residues (Arg 4 and Arg 5) with the catalytic binding pocket of the enzyme, and on the other hand, renders the phosphorylated serine readily accessible to the enzyme. This structure analysis provides an explanation for the unique substrate recognition of GSK-3. The design of small molecules that mimic the structure presented here thus provides a method for
25 obtaining potential selective inhibitors for GSK-3.

Chemical Syntheses:

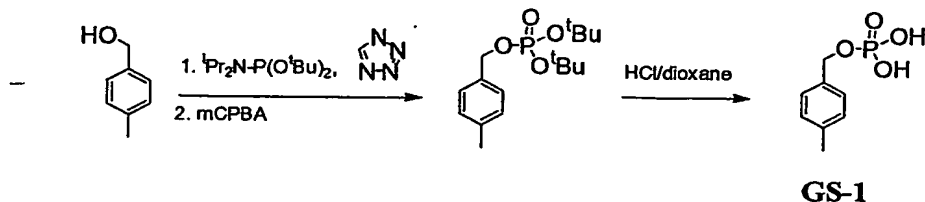
Synthesis of p-methyl benzyl phosphate (GS-1):

The general synthesis of GS-1 is depicted in Scheme 1 below.

30

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Scheme 1



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Preparation of di-tert-butyl, p-methyl benzyl phosphate: 1H-Tetrazole solution (0.45 M in acetonitrile, 20 ml, 9 mmol, 3 equivalents) was added in one portion to a stirred solution of 4-methylbenzyl alcohol (0.4 gram, 3.3 mmol, 1.1 equivalent) and di-tert-butyl diisopropyl phosphoramidite (0.95 ml, 0.83 gram, 3 mmol, 1 equivalent) in dry THF (3 ml). The mixture was stirred for 15 minutes at 20 °C. The mixture was cooled to -40 °C (by means of dry ice/acetonitrile), and a solution of 85 % m-chloroperbenzoic acid (mCPBA) (0.81 gram in 1 ml dichloromethane, 4 mmol, 1.3 equivalents) in dichloromethane (4 mL) was rapidly added while the reaction temperature was kept below 0 °C. The solution was allowed to warm up to room temperature and after stirring for 5 minutes at 20 °C, 10 % aqueous NaHSO₃ (10 ml) was added and the mixture was stirred for a further 10 minutes. The mixture was then extracted with ether (70 ml) and the aqueous phase discarded. The ethereal phase was washed with 10 % aqueous NaHSO₃ (2 x 20 ml), 5 % saturated aqueous NaHCO₃ (2 x 20 ml), dried on sodium sulfate and filtered. The organic filtrate was evaporated and the residue was purified by chromatography on a silica gel column, using a mixture of EtOAc/hexanes 1:15 as eluent, to give a mixture of the product (di-tert-butyl, p-methyl benzyl phosphate) and the starting material, which was used without further separation.

¹H NMR (200 MHz, CDCl₃): δ = 7.22 (m, 4H, Ar), 4.93 (d, J = 7.22 Hz, 2H, CH₂O), 2.33 (s, 3H, CH₃), 1.46 (s, 18H, OtBu).

¹³C NMR (50.4 MHz, CDCl₃): δ = 137.7 (Ar), 137.0 (Ar), 129.0 (Ar), 127.7 (Ar), 82.3 (c), 68.3 (CH₂O), 29.8 (OtBu), 21.1 (CH₃).

³¹P NMR (81.3 MHz, CDCl₃): δ = -9.4 ppm.

Preparation of p-methyl benzyl phosphate: A solution of HCl (4M in dioxane, 2 ml, 8 mmol, 2.6 equivalents) and dioxane (6 ml) was added to the obtained

di-tert-butyl, p-methyl benzyl phosphate at 20 °C and the reaction was monitored by TLC. Once the hydrolysis was completed, the dioxane was evaporated under reduced pressure, and the residue was dissolved in water (15 ml) and washed with ether (2 x 15 ml) to remove excess of the benzyl alcohol starting material. The solvent was then evaporated under reduced pressure and the resultant clear oil slowly changed into a colorless solid upon a prolonged high vacuum drying, to give 0.18 gram (30 %) of the final product.

^1H NMR (200 MHz, D_2O): δ = 7.27 (d, J = 8.1 Hz, 2H, Ar), 7.19 (d, J = 8.1 Hz, 2H, Ar), 4.82 (d, J = 7.0 Hz, 2H, CH_2O), 2.26 (s, 3H, CH_3).

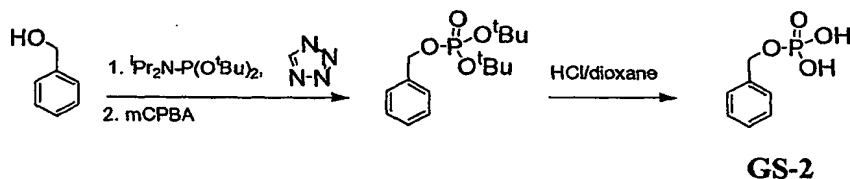
^{13}C NMR (50.4 MHz, D_2O): δ = 138.7 (Ar), 134.0 (Ar), 129.2 (Ar), 128.0 (Ar), 68.0 (CH_2O), 20.1 (CH_3).

^{31}P NMR (81.3 MHz, D_2O): δ = 0.6 ppm.

Synthesis of benzyl phosphate (GS-2):

The general synthesis of benzyl phosphate is depicted in Scheme 2 hereinbelow:

Scheme 2



Preparation of di-tert-butyl, benzyl phosphate: 1H-Tetrazole solution (0.45 M in acetonitrile 20 ml, 9 mmol, 3 equivalents) was added in one portion to a stirred solution of the benzyl alcohol (0.34 ml, 3.3 mmol, 1.1 equivalent) and di-tert-butyl diisopropyl phosphoramidite (0.95 ml, 0.83 gram, 3 mmol, 1 equivalent) in dry THF (3 ml). The mixture was stirred for 15 minutes at 20 °C and was thereafter cooled to -40 °C (by means of dry ice/acetonitrile). A solution of 85 % mCPBA (0.81 gram in 1 ml dichloromethane, 4 mmol, 1.3 equivalents) in dichloromethane (DCM) (4 ml) was rapidly added while the reaction temperature was kept below 0 °C. The solution was allowed to warm up to room temperature and after stirring for 5 minutes at 20 °C, 10 % aqueous NaHSO_3 (10 ml) was added and the mixture stirred for additional 10

50

minutes. The mixture was then extracted with ether (70 ml) and the aqueous phase discarded. The ethereal phase was washed with 10 % aqueous NaHSO_3 (2 x 20 ml), 5 % saturated aqueous NaHCO_3 (2 x 20 ml), dried over sodium sulfate and filtered. The organic layer was evaporated and the residue was purified by chromatography on a silica gel column using a mixture of EtOAc/hexanes 1:15 as eluent, to give a mixture of the product (di-tert-butyl, benzyl phosphate) and the starting material, which was used without further purification.

^1H NMR (200 MHz, CDCl_3): δ = 7.36 (m, 5H, Ar), 4.99 (d, J = 7.33 Hz, 2H, CH_2O), 1.46 (s, 18H, OtBu).

^{31}P NMR (81.3 MHz, CDCl_3): δ = -9.3 ppm.

Preparation of benzyl phosphate: A solution of HCl (4M in dioxane, 2 ml, 8 mmol, 2.6 equivalents) and dioxane (6 ml) was added to obtained di-tert-butyl, benzyl phosphate at 20 °C and the reaction was monitored by TLC. Once the hydrolysis was completed, the dioxane was evaporated under reduced pressure, and the residue was dissolved in water (15 ml) and washed with ether (2 x 15 ml) to remove excess of the benzyl alcohol starting material. The solvent was then evaporated under reduced pressure and the resultant clear oil slowly changed into a colorless solid upon a prolonged high vacuum drying, to give 0.17 gram (30 %) of the final product.

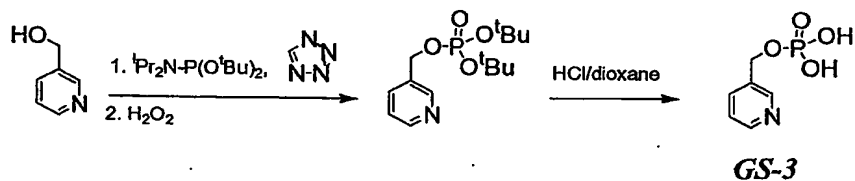
^1H NMR (200 MHz, D_2O): δ = 7.34 (m, 5H, Ar), 4.82 (d, J = 7.09 Hz, 2H, CH_2O).

^{31}P NMR (81.3 MHz, D_2O): δ = 0.7 ppm.

Synthesis of 3-pyridylmethyl phosphate (GS-3):

The general synthesis of 3-pyridylmethyl phosphate is depicted in Scheme 3 below:

Scheme 3



Preparation of di-tert-butyl, 3-Pyridylmethyl phosphate: 1H-Tetrazole solution (0.45 M in acetonitrile, 20 ml, 9 mmol, 3 equivalents) was added in one portion to a stirred solution of 3-pyridylmethanol (0.31 ml, 3.3 mmol, 1.1 equivalent) and di-tert-butyl diisopropyl phosphoramidite (0.95 ml, 0.83 gram, 3 mmol, 1 equivalent) in dry THF (3 ml). The mixture was stirred for 15 minutes at 20 °C and was thereafter cooled to -40 °C (by means of dry ice/acetonitrile). A solution of 85 % mCPBA (0.81 gram in 1 ml DCM, 4 mmol, 1.3 equivalents) in DCM (4 ml) was then rapidly added while the reaction temperature was kept below 0 °C. The solution was allowed to warm up to room temperature and after stirring for 5 minutes at 20 °C, 10% aqueous NaHSO₃ (10 ml) was added and the mixture was stirred for additional 10 minutes. The mixture was then extracted with ether (70 ml) and the aqueous phase discarded. The ethereal phase was washed with 10 % aqueous NaHSO₃ (2 x 20 ml), 5 % saturated aqueous NaHCO₃ (2 x 20 ml), dried over sodium sulfate and filtered. The organic filtrate was evaporated and the residue was purified by chromatography on a silica gel column using a mixture of CHCl₃/hexanes 1:1 as eluent, to give a mixture of di-tert-butyl, 3-Pyridylmethyl phosphate and the starting material, which was used without further purification.

¹H NMR (200 MHz, CDCl₃): δ = 8.52 (d, J = 1.56 Hz, 1H, Ar), 8.51 (dd, J = 4.83 Hz, J = 1.53 Hz, 1H, Ar), 7.80 (m, 1H, Ar), 7.33 (dd, J = 7.44 Hz, J = 4.62 Hz, 1H, Ar), 4.94 (d, J = 6.83 Hz, 2H, CH₂O), 1.46 (s, 18H, OtBu).

¹³C NMR (50.4 MHz, CDCl₃): δ = 148.2 (Ar), 148.1 (Ar), 135.3 (Ar), 134.3 (Ar), 123.1 (Ar), 77.9 (c), 64.1 (CH₂O), 29.6 (OtBu).

³¹P NMR (81.3 MHz, CDCl₃): δ = -9.4 ppm.

Preparation of 3-pyridylmethyl phosphate: A solution of HCl (4M in dioxane, 2 ml, 8 mmol, 2.6 equivalents) and dioxane (6 ml) was added to the obtained di-tert-butyl, 3-Pyridylmethyl phosphate at 20 °C and the reaction was monitored by TLC. Once the hydrolysis was completed, the dioxane was evaporated under reduced pressure, and the residue was dissolved in water (15 ml) and washed with ether (2 x 15 ml). The solvent was then evaporated under reduced pressure to give 0.19 gram (30 %) of the final product.

¹H NMR (400 MHz, D₂O): δ = 8.72 (t, J = 0.81 Hz, 1H, Ar), 8.62 (d, J = 5.71 Hz, 1H, Ar), 8.51 (d, J = 8.27 Hz, 1H, Ar), 7.96 (dd, J = 8.15 Hz, J = 5.94 Hz, Ar), 5.04 (d, J = 8.23 Hz, 2H, CH₂O).

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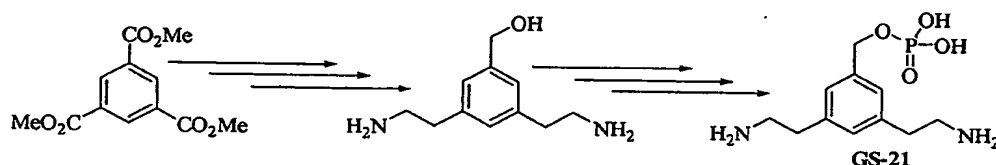
^{13}C NMR (100.8 MHz, D_2O): $\delta = 144.9$ (Ar), 139.9 (Ar), 139.0 (Ar), 126.8 (Ar), 62.8 (CH_2O).

^{31}P NMR (162 MHz, D_2O): $\delta = 0.76$ ppm.

Synthesis of 3,5-bis(2-aminoethyl)benzyl phosphate (GS-21):

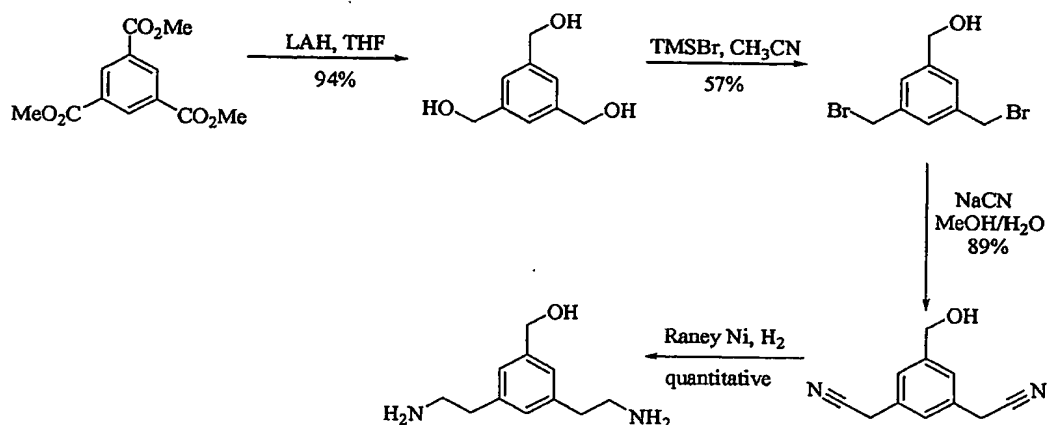
A general strategy for synthesizing GS-21, depicted in Scheme 4 below, as well as a purification protocol of the final product, were designed and practiced. 3,5-Bis(2-aminoethyl)benzyl phosphate (GS-21) was obtained in 5 % overall yield by an eight-step synthesis. The corresponding trifluoroacetic acid salt was also prepared.

Scheme 4



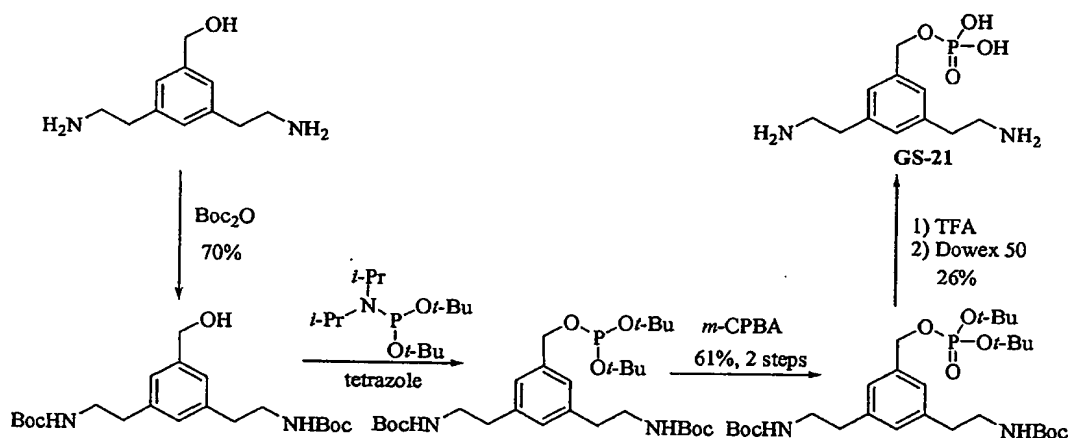
The benzyl alcohol intermediate (see, Scheme 4) was identified as a key intermediate obtainable in four steps from the inexpensive starting material trimethyl 1,3,5-benzenetricarboxylate, as is detailed hereinbelow and is depicted in Scheme 5.

Scheme 5



The phosphate moiety was then introduced by reaction of the alcohol with di-*tert*-butyl diisopropyl phosphoramidite, in the presence of tetrazole, according to the method of Johns (*Tetrahedron Lett.* 1988, 29, 2369–2372). Immediate oxidation without isolation of the resulting phosphite by *m*-chloroperbenzoic acid (mCPBA) yielded the corresponding phosphate ester. Global deprotection of the amines and the phosphate was achieved by the use of trifluoroacetic acid under controlled conditions. The material was then obtained as its trifluoroacetate salt. The latter was recrystallized prior to treatment with an ion-exchange resin to afford the desired product with adequate purity typically approximately 90 % (AUC by HPLC), as depicted in Scheme 6 below.

Scheme 6



Following is a detailed description of the synthesis:

Preparation of 1,3,5-Tris(hydroxymethyl)benzene: A 3-liter, round-bottom flask equipped with an overhead stirrer, an addition funnel and a reflux condenser was charged with lithium aluminum hydride (49.7 grams, 1.31 mol) and anhydrous THF (500 ml) under nitrogen atmosphere. The resulting suspension was slowly heated to reflux and a solution of trimethyl-1,3,5-benzenetricarboxylate (100.0 grams, 0.40 mol) in anhydrous THF (1.0 liter) was added dropwise thereto while maintaining a gentle reflux (3 hours). The resulting gray suspension was stirred under reflux for additional 7 hours and then cooled in an external ice-water bath. The excess lithium aluminum hydride was hydrolyzed by dropwise addition of water (50 ml, 45 minutes),

then 15 % NaOH (50 ml, slow stream), and finally more water (150 ml, slow stream). The resulting suspension was stirred at ambient temperature for 14 hours. The solids were filtered off and the filtrate was concentrated under high vacuum to obtain a colorless oil which slowly solidified to afford 1,3,5-tris(hydroxymethyl)benzene (62.3 grams, 94 %) as a white solid. The ^1H NMR and ^{13}C NMR spectra, presented in Figures 4a-b, were consistent with the assigned structure.

Preparation of 3,5-Bis(bromomethyl)benzyl Alcohol: A 2-liter, round-bottom flask equipped with a magnetic stir bar and an addition funnel was charged with 1,3,5-tris(hydroxymethyl)benzene [33.7 grams, 0.20 mol] and anhydrous acetonitrile (750 ml). To the resulting suspension was added, with stirring, bromotrimethylsilane (TMSBr) 979.0 ml, 0.60 mol) as a slow stream. The white slurry turned brown and viscous. The reaction mixture was then heated to 40 °C for 25 minutes and resulted in a clear solution. The reaction was judged to be complete by TLC analysis (90:10 methylene chloride/methanol, visualization by UV, starting material R_f 0.07, product R_f 0.77). The solvent was removed under reduced pressure to obtain a brown paste. The crude material was purified by column chromatography (silica gel, 0–5 % MeOH/ CH_2Cl_2). 3,5-Bis(bromomethyl)benzyl alcohol (33.5 grams, 57 %) was obtained as a white solid. The ^1H NMR and ^{13}C NMR spectra, presented in Figure 5a-b, were consistent with the assigned structure.

Preparation of 3,5-Bis(cyanomethyl)benzyl Alcohol: A 1-liter, round-bottom flask equipped with an overhead stirrer, an addition funnel and a reflux condenser was charged with 3,5-Bis(bromomethyl)benzyl alcohol (33.1 grams, 0.11 mol) and methanol (400 ml). The resulting clear solution was heated to reflux. A solution of sodium cyanide (16.2 grams, 0.33 mol) in water (25 ml) was added slowly. Heating was continued under reflux for 6 hours before the reaction was judged to be complete by TLC analysis (95:5 methylene chloride/methanol, visualization by UV, starting material R_f 0.62, product R_f 0.38). The reaction mixture was cooled to ambient temperature and solvent was removed under reduced pressure to obtain a brown paste. The latter was triturated with MTBE (6 × 100 ml). The MTBE extracts were combined and the solvent removed under reduced pressure. The yellow oil thus obtained was then purified by column chromatography (silica gel, 0–5 % MeOH/ CH_2Cl_2). 3,5-Bis(cyanomethyl)benzyl alcohol (18.7 grams, 89 %) was obtained as a light brown oil, which slowly turned into a waxy white solid. A 1-gram

sample was removed and purified via column chromatography to give a purified sample. The ^1H NMR and ^{13}C NMR spectra, presented in Figures 6a-b, were consistent with the assigned structure.

Preparation of 3,5-Bis(aminoethyl)benzyl Alcohol: A sample of 3,5-Bis(cyanomethyl)benzyl alcohol (8.0 grams, 0.04 mol) was divided in three parts and each 2.5- to 3.0-grams portion was charged into separate 500-ml Parr bottles, followed by ethanol (100 ml), and aqueous NaOH (1.2 grams in 5 ml of water). To the resulting solution was added Raney Ni (50 % suspension in water, 1.2 grams). The mixture was hydrogenated at 30 psi on a Parr shaker. The reaction was monitored by ^1H NMR and judged complete after 3 hours. The catalyst was filtered on a pad of diatomaceous earth and the diatomaceous earth pads washed with ethanol (200 ml). The filtrates from all three reactions were combined and solvent removed under reduced pressure to obtain 3,5-bis(aminoethyl)benzyl alcohol as a brown paste of (14.16 grams). ^1H NMR spectrum of the product, presented in Figure 7, shows presence of 25 % (w/w) of ethanol. No noticeable change in ethanol content was observed when the sample was dried under high vacuum for an extended period of time. This material was used in the next step of the synthesis without any further purification.

Preparation of 3,5-Bis(tert-butoxycarbonylaminoethyl)benzyl Alcohol: A three-neck, 3-liter, round-bottom flask equipped with a magnetic stir bar, thermometer and gas inlet adapter was charged with 3,5-bis(aminoethyl)-1-hydroxymethylbenzyl alcohol (29.4 grams) dissolved in THF (590 ml) and 2 N aqueous NaOH (590 ml). To the stirred mixture was added di-tert-butyl dicarbonate (59.4 grams, 272 mmol) in one portion. The mixture was heated to 45 °C for 4 hours. The resulting solution was cooled to ambient temperature and the volatile organics were removed by vacuum. To the resulting water mixture was added methanol (600 ml). The stirred solution was heated to 45 °C for 2 days in order to selectively hydrolyze the tert-butoxycarbonate moiety while preserving the carbamates. After cooling to ambient temperature the solution had volatiles removed and the aqueous mixture was extracted with chloroform (3 × 600 ml). The organic layers were combined, washed with brine (600 ml) and concentrated. After drying under high vacuum, crude 3,5-bis(tert-butoxycarbonylaminoethyl)benzyl alcohol (24.88 grams) was obtained in 67 % yield

as an off-white solid. The ^1H NMR spectrum, presented in Figure 8, was consistent with the assigned structure. The crude material was used without further purification.

Preparation of protected 3,5-Bis(2-aminoethyl)benzyl Phosphate: A 500-ml, round-bottom flask equipped with a magnetic stir bar and an addition funnel was charged with 3,5-bis(*tert*-butoxycarbonylaminoethyl)benzyl alcohol (2.3 grams, 0.0058 mol) and anhydrous methylene chloride (45 ml). The resulting solution was cooled to approximately 5 °C in an ice-water bath. A solution of di-*tert*-butyl diisopropylphosphoramidite (4.5 ml, 0.0144 mol) in anhydrous acetonitrile (45 ml) was added as a slow stream from the addition funnel. A solution of tetrazole (1.0 grams, 0.0144 mol) in a 1:1 mixture of anhydrous acetonitrile/anhydrous methylene chloride (90 ml) was then added slowly (15 minutes). The resulting white suspension was stirred at approximately 5 °C for 1 hour and the reaction was judged complete by TLC analysis (95:5 chloroform/isopropyl alcohol, visualization by staining in ninhydrin, starting material R_f 0.23, product R_f 0.30). The solvent was removed under reduced pressure to obtain a paste, which was dissolved in anhydrous methylene chloride (75 ml) and cooled in a dry ice/acetonitrile bath. A solution of mCPBA (1.3 grams, 0.0144 mol) in anhydrous methylene chloride (50 ml) was added all at once. The resulting mixture was stirred for 1 hour, allowed to warm up to ambient temperature (1 hour) and stirred for another 30 minutes. The reaction mixture then was washed successively with 1.0 M aqueous solution of sodium thiosulfate (100 ml) and saturated sodium bicarbonate (2 × 100 ml). The organic extract was dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure to obtain the crude phosphate as a yellow oil, which was then purified by column chromatography (silica gel, 0–5 % MeOH/ CH_2Cl_2). The protected 3,5-Bis(2-aminoethyl)benzyl phosphate (2.1 grams, 61 %) was obtained as a viscous, colorless oil. The ^1H NMR, ^{13}C NMR and ^{31}P NMR spectra thereof, presented in Figures 9a-c, were consistent with the assigned structure.

Preparation of 3,5-Bis(2-aminoethyl)benzyl Phosphate TFA Salt: A 250-ml, round-bottom flask was equipped with a magnetic stir bar was charged with the protected phosphate (2.9 grams, 0.0049 mol), anhydrous dichloromethane (30 ml) and trifluoroacetic acid (30 ml). The resulting clear solution was stirred at ambient temperature for 3 hours. The reaction was judged complete by ^1H NMR and ^{31}P NMR analysis. Removal of the solvent under reduced pressure afforded a viscous

orange oil, which was dissolved in methanol (7.5 ml) and added with stirring to diethyl ether (500 ml), resulting in precipitation of the product. The resulting slurry was stirred for 1 hour at ambient temperature and then solids allowed to settle. The clear solution was decanted off from the top and the product triturated with ether (2 × 100 ml). Each time the solids were allowed to settle and the clear solution was decanted off. The product was finally dried in a vacuum oven for 108 hours at 55 °C and then for an additional 192 hours at 65 °C to afford 3,5-bis(2-aminoethyl)benzyl phosphate TFA salt (1.78 grams, 71 %) as a white solid. The ¹H NMR, ¹³C NMR and ³¹P NMR spectra, presented in Figures 10a-c were consistent with the assigned structure. The ¹H NMR spectrum showed the presence of 8.5 % (w/w) of ether in the product. This ether proved very difficult to remove and the material was characterized as hygroscopic. Mass spectrum of the product, presented in Figure 10d, indicated a molecular peak at *m/z* 275 [C₁₁H₁₉N₂O₄P + H]⁺.

Preparation of 3,5-Bis(2-aminoethyl)benzyl Phosphate (GS-21): A three-neck, 5-liter, round-bottom flask equipped with an overhead mechanical stirrer, thermometer, 1-liter pressure-equalizing addition funnel and gas inlet adapter was charged with a solution of 3,5-bis(*tert*-butoxycarbonylaminoethyl)benzyl alcohol (24.88 grams, 63.15 mmol) in anhydrous dichloromethane (1 l) under nitrogen atmosphere. The reaction mixture was cooled with an ice/brine bath. Di-*tert*-butyl diisopropylphosphoramidite (49.8 ml, 157.9 mmol) in anhydrous acetonitrile (1 liter) was added via the pressure-equalizing addition funnel at such a rate that the reaction temperature was maintained <6 °C. Tetrazole (351 ml of a 0.45 M solution in acetonitrile, 157.9 mmol) was diluted with anhydrous acetonitrile (150 ml) and anhydrous dichloromethane (500 ml) and added via the pressure-equalizing addition funnel at such a rate that the temperature was maintained under 6 °C. After the addition was completed, the flask was left in the cold bath and the reaction mixture stirred for 1 hour. The flask was then cooled to -35 °C by means of dry ice/acetonitrile bath. A solution of 3-chloroperoxy-benzoic acid (18.4 grams, 82.1 mmol) in anhydrous dichloromethane (500 ml) was added in one portion. The mixture was allowed to warm to ambient temperature and thereafter stirred for 2 hours. The solution was poured into a solution of Na₂S₂O₃ (20 grams) and K₂CO₃ (50 grams) in water (1.5 liters). The resulting biphasic mixture had a pH of 11. After stirring for 15 minutes the volatile organics were removed in vacuum and the water

layer was extracted with chloroform (4 × 750 ml). The combined organic layers were dried over magnesium sulfate, filtered and concentrated to a yellow oil (69 grams). The crude material was purified by column chromatography (silica gel, MTBE/heptane, 6:4). Mixed fractions containing the product were combined and concentrated to a light yellow oil (32.6 grams). A 28.6-grams portion of the oil was dissolved into dichloromethane (287 ml, 10 volumes) and charged into a 1-liter, round-bottom flask equipped with a 500-ml pressure-equalizing addition funnel and magnetic stir bar. Trifluoroacetic acid (287 ml, 10 volumes) was added rapidly via the pressure-equalizing addition funnel. The resulting solution was stirred for 5 hours. After concentrating and drying overnight under high vacuum, a thick orange oil (37.88 grams) was obtained. The residue was dissolved in water (57 ml, 1.5 volumes) and added dropwise into stirred methanol (90 volumes) yielding a precipitate. After stirring for 30 minutes, the solids were allowed to settle for 1 hour and the liquid was decanted off. The remaining liquid was removed in vacuum giving 13.72 grams of solid. The material was dissolved in water (68 ml, 5 volumes) and loaded onto Dowex 50WX8-200 ion-exchange resin (137 grams). The column was washed with water (550 ml, 40 volumes). The product was eluted with 3:1 MeOH/aqueous NH₄OH (2 liters, 145 volumes). The methanol fractions were concentrated under reduced pressure to yield an off-white solid. The solid was dissolved in a minimum amount of water and added into stirred methanol (40 volumes). The precipitate was collected via filtration and dried overnight under vacuum. The resulting powder was triturated with water (7 volumes). After filtration and drying under high vacuum, the final product (2.0 grams) was obtained as a white powder. The filtrate was concentrated and the residue triturated with water (5 volumes). After filtration and drying under high vacuum, a second crop of product [0.9 grams] was obtained. The two lots were combined and blended for 10 minutes. 3,5-Bis(2-aminoethyl)benzyl phosphate (GS-21) was obtained as a white powder. The ¹H NMR, ³¹P NMR, and ¹³C NMR spectra of the product, presented in Figures 11a-c, were consistent with the assigned structure. The MS spectrum, presented in Figure 11d, indicated a molecular peak at 275 [C₁₁H₁₉N₂O₄P + H]⁺. HPLC chromatogram (obtained using method A described above), presented in Figure 11e, showed a 96.7 % purity of the product. The final product was characterized as non-hygroscopic.

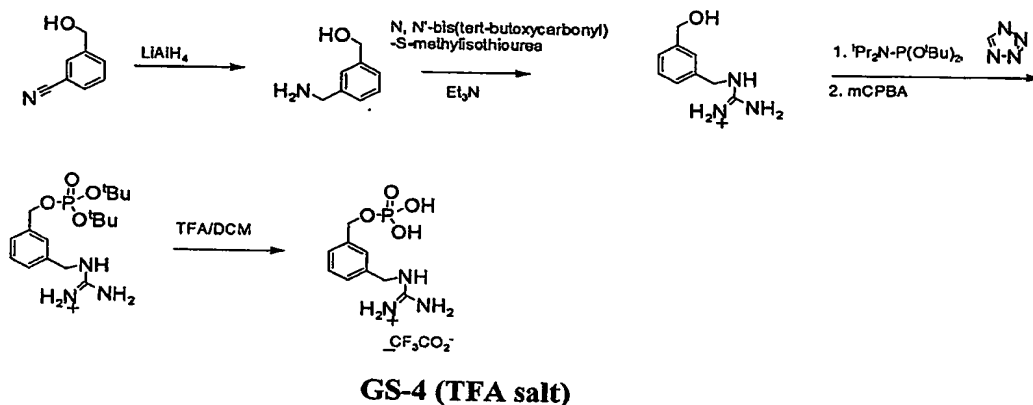
Using the same strategy, the novel compounds GS-4 and GS-5 were synthesized as follows:

Synthesis of 3-(guanidinomethyl) benzyl phosphate (GS-4):

The general synthesis of GS-4, as its trifluoroacetic acid salt, is depicted in Scheme 7 below:

5

Scheme 7



Preparation of 3-(aminomethyl)benzyl alcohol: A solution of 3-(hydroxymethyl)benzonitrile in THF was slowly added to a refluxing solution of LiAlH_4 in THF with vigorous stirring, maintained under nitrogen atmosphere. The solution was heated at reflux overnight and water was thereafter slowly added dropwise to quench the reaction (until no further evolution of H_2 was apparent). The THF was evaporated under reduced pressure and ether/acidified water was added. The ether phase was discarded. The aqueous phase was washed with ether and the organic phase was discarded. NaOH was added until the pH of the aqueous phase reached pH 7. The solution was extracted with THF three times, dried over MgSO_4 and evaporated under reduced pressure to furnish a slightly yellow residue which was purified by chromatography on a silica gel column using a gradient eluent starting from ethyl acetate and ending with a mixture of 1:1 ethyl acetate:MeOH, to give the intermediate in a 40 % yield.

^1H NMR (200 MHz, $\text{d}^6\text{-DMSO}$): δ = 7.16-7.27 (m, 4H, Ar), 4.47 (s, 2H, CH_2O), 3.94 (bs, 2H, NH_2), 3.72 (s, 2H, CH_2NH_2).

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^{13}C NMR (50.4 MHz, CDCl_3) δ = 143.1, 142.8, 128.2, 125.9, 125.7, 124.9, 63.3, 45.6.

Preparation of 3-(N,N'-bis-BOC-guanidinomethyl) benzyl alcohol: A solution of 3-(aminomethyl)benzylalcohol, 3-(N,N'-bis(tert-butoxycarbonyl)-S-methylisothiourea and triethyl amine in dry DMF was stirred at room temperature overnight. An ether/water mixture was then added and the organic layer separated, while the aqueous layer was extracted with ether. The combined organic extract was washed with water, dried over MgSO_4 and evaporated under reduced pressure. The crude product was purified by flash chromatography using a gradient eluent starting from hexanes and ending with a mixture of 1:5 ethyl acetate: hexanes), in 85 % yield.

^1H NMR (200MHz, CDCl_3): δ = 11.51 (bs, 1H), 8.56 (bs, 1H), 7.22-7.38 (m, 4H), 4.70 (s, 2H), 4.65 (d, $J=5.1\text{Hz}$, 2H), 1.52 (s, 9H), 1.48 (s, 9H).

5 ^{13}C NMR (50 MHz, CDCl_3): δ = 155.9, 153.1, 141.5, 137.7, 128.9, 127.0, 126.4, 126.2, 65.0, 45.0, 28.2, 27.9.

Preparation of Di-tert-butyl, 3-(N,N'-bis-BOC-guanidinomethyl) benzyl phosphate: 1H-Tetrazole solution (0.45 M in acetonitrile, 20 ml, 9 mmol, 3 equivalents) was added in one portion to a stirred solution of 3-(N, N'-bis-BOC-guanidinomethyl)benzyl alcohol (1 equivalent) and di-tert-butyl diisopropyl phosphoramidite (1.42 ml, 1.24 grams, 4.5 mmol, 1.5 equivalents) in dry THF (3 ml). The mixture was stirred for 30 minutes at 20 °C and was then cooled to -40 °C (by means of dry ice/acetonitrile). A solution of 85 % mCPBA (1.25 grams in 1.5 ml DCM, 6.15 mmol, 2.0 equivalents) in DCM (4 ml) was rapidly added while keeping the reaction temperature below 0 °C. The solution was allowed to warm up to room temperature and, after stirring for 20 minutes, 10 % aqueous NaHSO_3 (10 ml) was added and the mixture was stirred for additional 5 minutes. The mixture was extracted with ether (70 ml) and the aqueous phase discarded. The ethereal phase was washed with 10 % aqueous NaHSO_3 (2 x 20 ml) and saturated aqueous NaHCO_3 (2 x 20 ml), dried over sodium sulfate and filtered. The organic filtrate was evaporated and the residue was purified by chromatography on a silica gel column using a gradient eluent of ethyl acetate/hexanes 1:9 to 1:5), to give a mixture of the phosphate ester product and the benzyl alcohol starting material, which was further purified by chromatography on a silica gel column, using a gradient eluent of

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CHCl_3 :MeOH 30:1 to 20:1), to give pure di-tert-butyl, 3-(N,N'-bis-BOC-guanidinomethy) benzyl phosphate in 70 % yield.

^1H NMR (200 MHz, CDCl_3): δ = 11.52 (bs, 1H), 8.53 (bs, 1H), 7.25-7.35 (m, 4H), 4.99 (d, J =7.2 Hz, 2H), 4.63 (d, J =5.1Hz, 2H), 1.51 (s, 9H), 1.47 (s, 27).

^{31}P NMR (162 MHz, CDCl_3): δ = -9.3.

Preparation of 3-(guanidinomethy) benzyl phosphate, trifluoroacetic acid salt: A solution of 25 % trifluoroacetic acid (TFA) in DCM was added to di-tert-butyl, 3-(N,N'-bis-BOC-guanidinomethy) benzyl phosphate at 20 °C and the reaction mixture was stirred for 18 hours. The solvent and TFA were thereafter evaporated under reduced pressure, and the residue was dissolved in water and washed with ether. The solvent was then evaporated under reduced pressure, to give the pure product in 60 % yield.

^1H NMR (200 MHz, D_2O): δ = 7.25-7.35 (m, 4H), 4.84 (d, J =7.2Hz, 2H), 4.37 (s, 1H).

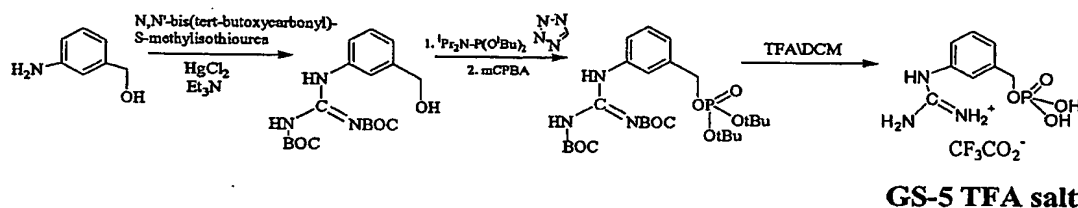
^{31}P NMR (162 MHz, CDCl_3): δ = 0.85.

^{19}F NMR: δ = -76.6.

Synthesis of 3-guanidinobenzyl phosphate (GS-5):

The general synthesis of GS-5, as its trifluoroacetic acid salt, is depicted in Scheme 8 below:

Scheme 8



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Preparation of 3-(N,N'-bis-BOC-guanidino)benzyl alcohol: A solution of N,N'-bis(tert-butoxycarbonyl)-S-methylisothiourea (1.32 gram, 4.4 mmol, 1.1 equivalents), mercury chloride (1.22 gram, 4.4 mmol, 1.1 equivalents) and triethylamine (1.72 ml, 12 mmol, 3 equivalents) was added to 3-aminobenzyl alcohol (0.5 gram, 4 mmol, 1.0 equivalent) in dry dimethylformamide (DMF) and the reaction

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mixture was stirred at room temperature for 5 hours. The mixture was thereafter extracted with ether/water and the organic layer was washed with saturated aqueous NH_4Cl and brine. The aqueous layer was extracted with ether. The combined ether solution was dried over MgSO_4 and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel using a gradient eluent of hexanes to 40:60 ethyl acetate:hexanes), to give the intermediate in 60 % yield.

^1H NMR (200 MHz, CDCl_3): δ = 11.60 (brs, 1H), 10.30 (brs, 1H), 7.11-7.55 (m, 4H), 4.65 (s, 2H), 1.49 (s, 9H), 1.48 (s, 9H).

^{13}C NMR (400 MHz, CDCl_3): δ = 171.4, 163.4, 153.7, 142.0, 136.7, 129.0, 123.4, 121.4, 120.8, 65.8, 64.7, 28.1, 27.9.

Preparation of di-tert-butyl, 3-(*N,N'*-bis-BOC-guanidino)benzyl phosphate:
1-H-tetrazole solution (0.45 M in acetonitrile, 18.4 ml, 8.3 mmol, 3 equivalents) was added in one portion to a stirred solution of 3-(*N,N'*-bis-BOC guanidino)benzyl alcohol (1 gram, 2.8 mmol, 1 equivalent) and di-tert-butyl diisopropyl phosphoramidite (1.13 ml, 3.6 mmol, 1.3 equivalents) in dry THF (3 ml). The mixture was stirred for 30 minutes at 20 °C and thereafter cooled to -40 °C (by means of dry ice/acetonitrile). A solution of 85 % mCPBA (0.85 gram in 1.5 ml DCM, 4.20 mmol, 1.5 equivalents) in DCM (4 ml) was rapidly added while keeping the reaction temperature below 0 °C. The reaction was allowed to reach room temperature and after stirring for 20 minutes, 10 % aqueous NaHSO_3 (10 ml) was added and the mixture stirred for additional 10 minutes. The mixture was extracted with ether (50 ml) and the aqueous phase discarded. The ethereal phase was washed with 10 % aqueous NaHSO_3 (2 x 20 ml) and saturated aqueous NaHCO_3 (2 x 20 ml), dried over MgSO_4 and filtered. The solvent was evaporated and the residue was purified by chromatography on a silica gel column using a gradient eluent of ethyl acetate/hexanes 10:90 to 30:70), to give the protected product in 60 % yield.

^1H NMR (200 MHz, CDCl_3): δ = 11.65 (brs, 1H), 10.40 (brs, 1H), 7.10-7.64 (m, 4H), 4.97 (d, $J=7.0\text{Hz}$, 2H), 1.49 (s, 18H), 1.45 (s, 18H).

^{13}C NMR (400 MHz, CDCl_3): δ = 153.3, 136.6, 129.2, 124.3, 122.1, 121.3, 67.9, δ 29.8, 28.0.

^{31}P NMR(200 MHz, CDCl_3): δ = -9.3.

Preparation of 3-guanidinobenzyl phosphate, trifluoroacetic acid salt: A solution of 25 % TFA (1.5 ml) in DCM (4.5 ml) was added to di-tert-butyl-3-(*N,N'*-

bis-BOC guanidino)benzyl phosphate (0.3 gram, 0.54 mmol, 1 equivalent) at 20 °C and the reaction mixture was stirred for 18 hours. The solvent and TFA were thereafter evaporated under reduce pressure and the residue was dissolved in water and washed with ether. The solvent was evaporated under reduced pressure
5 (lyophilizer), to give the pure product in 40 % yield ($C_{10}H_{13}F_3N_3O_6P$; Mw = 359.2 grams/mol).

1H NMR (200 MHz, $CDCl_3$): δ = 7.13-7.38 (m, 4H), 4.83 (d, J=7.6Hz, 2H)

^{13}C NMR (400 MHz, $CDCl_3$): δ = 156.3, 139.5, 134.3, 130.1, 126.8, 125.3, 124.6, 66.4.

10 ^{31}P NMR (200 MHz, $CDCl_3$): δ = 0.8.

In vitro inhibition assays:

In a preliminary inhibition assay, the GSK-3 inhibition activity of the known compounds phenyl phosphate, pyridoxal phosphate, GS-1, GS-2 and GS-3 was tested as described hereinabove. The results, presented in Figure 12 indicate that all the
15 tested compounds exerted an inhibition activity toward GSK-3, with the phosphate derivatives of pyridine, namely, pyridoxal phosphate and GS-3, being more active than the phosphate derivatives of phenyl (phenyl phosphate, GS-1 and GS-2).

In an additional inhibition assay, the GSK-3 inhibition activity of GS-1, GS-2, GS-3, GS-5 and GS-21 was tested. The ability of GSK-3 to phosphorylate PGS-1
20 peptide substrate was measured in the presence of indicated concentrations of these compounds. The results, presented in Figure 13, represent the percentage of GSK-3 activity as compared with a control incubation without inhibitors and are mean of 2 independent experiments \pm SEM, where each point was assayed in triplicate.

As is shown in Figure 13, all the tested compounds were found highly active
25 in inhibiting GSK-3 activity (IC_{50} values of 1-5 mM), with GS-3 and GS-5 being the most active compounds. These results may suggest that the presence of one or more nitrogen atoms in the ring or at an adjacent position thereto (e.g., directly attached to a ring atom) is a feature that may affect (enhance) the GSK-3 inhibition activity of newly designed small molecules.

30 ***Glucose Uptake:***

The ability of the newly designed compounds GS-5 and GS-21 to promote glucose uptake was tested in mouse primary adipocytes as described hereinabove. The relative [3H] 2-deoxy glucose incorporation observed in non-treated adipocytes

was normalized to 1 unit and the values obtained for [^3H] 2-deoxy glucose in adipocytes treated with GS-5 or GS-21 are presented as fold activation over cells treated with the peptide control, and are the mean of 6 independent experiments \pm SEM, where each point was assayed in triplicate.

5 The results, presented in Figures 14a (GS-5) and Figure 14b (GS-21) show that GS-21, at concentrations of 5 μM and 0.5 μM increased glucose uptake by 2.5-fold and 1.7 fold, respectively. A somewhat reduced effect was observed in the presence of GS-5, which enhanced glucose uptake approximately by 2-fold at a concentration of 10 μM . As is further shown in Figures 14a and 14b, the activation of
10 glucose uptake achieved by GS-5 and GS-21 was comparable to that achieved in the presence of 100 nM insulin. These results further demonstrate the ability of these newly designed compounds to act as insulin mimetics in potentiating insulin signaling and treating GSK-3 mediated disorders such as diabetes.

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Table 2

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ATOM	88	HD2	ARG	5	-0.202	2.651	-2.445	1.00	0.00
ATOM	89	NE	ARG	5	-2.008	1.659	-2.965	1.00	0.00
ATOM	90	HE	ARG	5	-2.795	2.241	-2.903	1.00	0.00
ATOM	91	CZ	ARG	5	-1.977	0.695	-3.845	1.00	0.00
ATOM	92	NH1	ARG	5	-0.857	0.392	-4.441	1.00	0.00
ATOM	93	HH11	ARG	5	-0.022	0.898	-4.225	1.00	0.00
ATOM	94	HH12	ARG	5	-0.834	-0.347	-5.115	1.00	0.00
ATOM	95	NH2	ARG	5	-3.067	0.035	-4.127	1.00	0.00
ATOM	96	HH21	ARG	5	-3.925	0.267	-3.670	1.00	0.00
ATOM	97	HH22	ARG	5	-3.043	-0.704	-4.801	1.00	0.00
ATOM	98	C	ARG	5	-2.236	0.954	2.575	1.00	0.00
ATOM	99	O	ARG	5	-2.260	-0.078	3.214	1.00	0.00
ATOM	100	N	PRO	6	-3.068	1.946	2.758	1.00	0.00
ATOM	101	CA	PRO	6	-4.149	1.876	3.807	1.00	0.00

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ATOM	102	HA	PRO	6	-3.740	1.628	4.790	1.00	0.00
ATOM	103	CB	PRO	6	-4.710	3.304	3.802	1.00	0.00
ATOM	104	HB1	PRO	6	-4.213	3.918	4.537	1.00	0.00
ATOM	105	HB2	PRO	6	-5.780	3.296	3.976	1.00	0.00
ATOM	106	CG	PRO	6	-4.411	3.814	2.426	1.00	0.00
ATOM	107	HG1	PRO	6	-4.357	4.887	2.428	1.00	0.00
ATOM	108	HG2	PRO	6	-5.177	3.477	1.740	1.00	0.00
ATOM	109	CD	PRO	6	-3.087	3.236	2.027	1.00	0.00
ATOM	110	HD2	PRO	6	-3.044	3.093	0.948	1.00	0.00
ATOM	111	HD1	PRO	6	-2.275	3.877	2.353	1.00	0.00
ATOM	112	C	PRO	6	-5.282	0.893	3.432	1.00	0.00
ATOM	113	O	PRO	6	-6.393	1.035	3.902	1.00	0.00
ATOM	114	N	SER	7	-5.030	-0.093	2.607	1.00	0.00
ATOM	115	HN	SER	7	-4.145	-0.207	2.235	1.00	0.00
ATOM	116	CA	SER	7	-6.110	-1.051	2.233	1.00	0.00
ATOM	117	HA	SER	7	-5.833	-1.603	1.348	1.00	0.00
ATOM	118	CB	SER	7	-6.238	-2.001	3.415	1.00	0.00
ATOM	119	HB1	SER	7	-6.552	-2.972	3.057	1.00	0.00
ATOM	120	HB2	SER	7	-6.974	-1.619	4.102	1.00	0.00
ATOM	121	OG	SER	7	-4.984	-2.104	4.077	1.00	0.00
ATOM	122	HG	SER	7	-5.045	-2.814	4.720	1.00	0.00
ATOM	123	C	SER	7	-7.430	-0.316	2.010	1.00	0.00
ATOM	124	O	SER	7	-8.251	-0.211	2.899	1.00	0.00
ATOM	125	N	TYR	8	-7.643	0.184	0.831	1.00	0.00
ATOM	126	HN	TYR	8	-6.966	0.078	0.127	1.00	0.00
ATOM	127	CA	TYR	8	-8.925	0.904	0.559	1.00	0.00
ATOM	128	HA	TYR	8	-9.535	0.924	1.451	1.00	0.00
ATOM	129	CB	TYR	8	-8.533	2.329	0.179	1.00	0.00
ATOM	130	HB1	TYR	8	-9.278	2.738	-0.498	1.00	0.00
ATOM	131	HB2	TYR	8	-7.570	2.317	-0.324	1.00	0.00
ATOM	132	CG	TYR	8	-8.466	3.172	1.458	1.00	0.00
ATOM	133	CD1	TYR	8	-7.422	4.091	1.648	1.00	0.00
ATOM	134	HD1	TYR	8	-6.664	4.205	0.901	1.00	0.00
ATOM	135	CD2	TYR	8	-9.451	3.035	2.465	1.00	0.00
ATOM	136	HD2	TYR	8	-10.261	2.331	2.352	1.00	0.00
ATOM	137	CE1	TYR	8	-7.360	4.861	2.815	1.00	0.00
ATOM	138	HE1	TYR	8	-6.553	5.566	2.952	1.00	0.00
ATOM	139	CE2	TYR	8	-9.379	3.809	3.629	1.00	0.00
ATOM	140	HE2	TYR	8	-10.134	3.702	4.394	1.00	0.00
ATOM	141	CZ	TYR	8	-8.336	4.721	3.803	1.00	0.00
ATOM	142	OH	TYR	8	-8.270	5.483	4.951	1.00	0.00
ATOM	143	HH	TYR	8	-7.345	5.662	5.136	1.00	0.00
ATOM	144	C	TYR	8	-9.680	0.230	-0.587	1.00	0.00
ATOM	145	O	TYR	8	-9.400	0.456	-1.747	1.00	0.00
ATOM	146	N	ARG	9	-10.639	-0.592	-0.266	1.00	0.00
ATOM	147	HN	ARG	9	-10.848	-0.751	0.681	1.00	0.00
ATOM	148	CA	ARG	9	-11.423	-1.283	-1.334	1.00	0.00
ATOM	149	HA	ARG	9	-11.870	-0.561	-1.999	1.00	0.00
ATOM	150	CB	ARG	9	-10.408	-2.140	-2.099	1.00	0.00
ATOM	151	HB1	ARG	9	-9.528	-1.554	-2.314	1.00	0.00
ATOM	152	HB2	ARG	9	-10.849	-2.477	-3.027	1.00	0.00
ATOM	153	CG	ARG	9	-10.015	-3.354	-1.253	1.00	0.00
ATOM	154	HG1	ARG	9	-10.141	-3.120	-0.206	1.00	0.00
ATOM	155	HG2	ARG	9	-8.982	-3.606	-1.444	1.00	0.00
ATOM	156	CD	ARG	9	-10.907	-4.543	-1.618	1.00	0.00
ATOM	157	HD1	ARG	9	-11.932	-4.342	-1.351	1.00	0.00
ATOM	158	HD2	ARG	9	-10.556	-5.439	-1.125	1.00	0.00
ATOM	159	NE	ARG	9	-10.781	-4.677	-3.096	1.00	0.00
ATOM	160	HE	ARG	9	-11.439	-4.252	-3.684	1.00	0.00
ATOM	161	CZ	ARG	9	-9.794	-5.361	-3.607	1.00	0.00

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ATOM	162	NH1	ARG	9	-8.770	-5.684	-2.865	1.00	0.00
ATOM	163	HH11	ARG	9	-8.742	-5.408	-1.904	1.00	0.00
ATOM	164	HH12	ARG	9	-8.014	-6.208	-3.256	1.00	0.00
ATOM	165	NH2	ARG	9	-9.831	-5.723	-4.860	1.00	0.00
ATOM	166	HH21	ARG	9	-10.615	-5.476	-5.429	1.00	0.00
ATOM	167	HH22	ARG	9	-9.074	-6.247	-5.252	1.00	0.00
ATOM	168	C	ARG	9	-12.504	-2.167	-0.705	1.00	0.00
ATOM	169	OT1	ARG	9	-13.492	-2.425	-1.372	1.00	0.00
ATOM	170	OT2	ARG	9	-12.324	-2.570	0.433	1.00	0.00

END

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Table 3

REMARK FILENAME="refine_1_20.pdb"

REMARK

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REMARK overall,bonds,angles,improper,vdw,noe,cdih

REMARK energies: 104.733, 3.47295, 70.7767, 3.51384, 6.64866,
20.3204, \$CDIH

REMARK

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REMARK bonds,angles,impropers,noe,cdih

REMARK rms-d: 4.442149E-03,1.21652,0.496579,7.90724E-02,0

REMARK

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REMARK noe, cdih

REMARK violations.: 0, 0

REMARK

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REMARK DATE:03-Apr-00 08:41:00 created by user: orish

ATOM	1	CA	ILE	1B	-9.783	-1.457	-0.558	1.00	0.00
ATOM	2	HA	ILE	1B	-9.677	-0.665	-1.298	1.00	0.00
ATOM	3	CB	ILE	1B	-11.259	-1.637	-0.199	1.00	0.00
ATOM	4	HB	ILE	1B	-11.578	-0.796	0.417	1.00	0.00
ATOM	5	CG1	ILE	1B	-11.441	-2.945	0.598	1.00	0.00
ATOM	6	HG11	ILE	1B	-12.251	-2.816	1.316	1.00	0.00
ATOM	7	HG12	ILE	1B	-10.519	-3.167	1.135	1.00	0.00
ATOM	8	CG2	ILE	1B	-12.101	-1.660	-1.481	1.00	0.00
ATOM	9	HG21	ILE	1B	-12.492	-0.662	-1.677	1.00	0.00
ATOM	10	HG22	ILE	1B	-12.930	-2.357	-1.358	1.00	0.00
ATOM	11	HG23	ILE	1B	-11.480	-1.978	-2.318	1.00	0.00
ATOM	12	CD1	ILE	1B	-11.776	-4.119	-0.334	1.00	0.00
ATOM	13	HD11	ILE	1B	-11.998	-5.004	0.263	1.00	0.00
ATOM	14	HD12	ILE	1B	-10.926	-4.325	-0.983	1.00	0.00
ATOM	15	HD13	ILE	1B	-12.644	-3.866	-0.941	1.00	0.00
ATOM	16	C	ILE	1B	-8.973	-1.137	0.677	1.00	0.00
ATOM	17	O	ILE	1B	-9.510	-0.787	1.709	1.00	0.00
ATOM	18	N	ILE	1B	-9.351	-2.764	-1.130	1.00	0.00
ATOM	19	HT1	ILE	1B	-8.379	-2.681	-1.489	1.00	0.00
ATOM	20	HT2	ILE	1B	-9.987	-3.028	-1.910	1.00	0.00
ATOM	21	HT3	ILE	1B	-9.383	-3.494	-0.391	1.00	0.00
ATOM	22	N	LEU	2	-7.676	-1.250	0.593	1.00	0.00
ATOM	23	HN	LEU	2	-7.221	-1.531	-0.230	1.00	0.00
ATOM	24	CA	LEU	2	-6.745	-0.970	1.725	1.00	0.00
ATOM	25	HA	LEU	2	-7.286	-0.659	2.617	1.00	0.00
ATOM	26	CB	LEU	2	-6.051	-2.305	1.992	1.00	0.00
ATOM	27	HB1	LEU	2	-5.606	-2.675	1.069	1.00	0.00
ATOM	28	HB2	LEU	2	-6.782	-3.027	2.359	1.00	0.00
ATOM	29	CG	LEU	2	-4.955	-2.110	3.041	1.00	0.00
ATOM	30	HG	LEU	2	-5.142	-1.190	3.595	1.00	0.00
ATOM	31	CD1	LEU	2	-4.955	-3.296	4.007	1.00	0.00
ATOM	32	HD11	LEU	2	-5.261	-2.958	4.997	1.00	0.00
ATOM	33	HD12	LEU	2	-3.952	-3.720	4.062	1.00	0.00
ATOM	34	HD13	LEU	2	-5.651	-4.055	3.651	1.00	0.00
ATOM	35	CD2	LEU	2	-3.595	-2.020	2.345	1.00	0.00
ATOM	36	HD21	LEU	2	-3.732	-1.671	1.322	1.00	0.00
ATOM	37	HD22	LEU	2	-3.127	-3.004	2.334	1.00	0.00
ATOM	38	HD23	LEU	2	-2.956	-1.320	2.884	1.00	0.00
ATOM	39	C	LEU	2	-5.725	0.080	1.347	1.00	0.00
ATOM	40	O	LEU	2	-4.915	0.491	2.155	1.00	0.00
ATOM	41	N	SER	3	-5.747	0.528	0.122	1.00	0.00

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ATOM	42	HN	SER	3	-6.390	0.212	-0.547	1.00	0.00
ATOM	43	CA	SER	3	-4.806	1.562	-0.398	1.00	0.00
ATOM	44	HA	SER	3	-4.771	1.557	-1.486	1.00	0.00
ATOM	45	CB	SER	3	-5.388	2.889	0.083	1.00	0.00
ATOM	46	HB1	SER	3	-6.272	3.133	-0.510	1.00	0.00
ATOM	47	HB2	SER	3	-4.648	3.677	-0.034	1.00	0.00
ATOM	48	OG	SER	3	-5.738	2.778	1.457	1.00	0.00
ATOM	49	HG	SER	3	-6.250	3.554	1.696	1.00	0.00
ATOM	50	C	SER	3	-3.416	1.369	0.164	1.00	0.00
ATOM	51	O	SER	3	-3.131	1.747	1.283	1.00	0.00
ATOM	52	N	ARG	4	-2.534	0.782	-0.597	1.00	0.00
ATOM	53	HN	ARG	4	-2.747	0.466	-1.515	1.00	0.00
ATOM	54	CA	ARG	4	-1.116	0.522	-0.175	1.00	0.00
ATOM	55	HA	ARG	4	-0.840	1.104	0.716	1.00	0.00
ATOM	56	CB	ARG	4	-1.095	-0.975	0.176	1.00	0.00
ATOM	57	HB1	ARG	4	-1.739	-1.526	-0.506	1.00	0.00
ATOM	58	HB2	ARG	4	-1.453	-1.114	1.200	1.00	0.00
ATOM	59	CG	ARG	4	0.323	-1.521	0.077	1.00	0.00
ATOM	60	HG1	ARG	4	1.018	-0.714	-0.142	1.00	0.00
ATOM	61	HG2	ARG	4	0.369	-2.273	-0.711	1.00	0.00
ATOM	62	CD	ARG	4	-0.681	-2.146	1.415	1.00	0.00
ATOM	63	HD1	ARG	4	-0.203	-2.604	1.849	1.00	0.00
ATOM	64	HD2	ARG	4	1.067	-1.373	2.079	1.00	0.00
ATOM	65	NE	ARG	4	1.715	-3.169	1.096	1.00	0.00
ATOM	66	HE	ARG	4	2.519	-3.100	1.652	1.00	0.00
ATOM	67	CZ	ARG	4	1.576	-4.075	0.168	1.00	0.00
ATOM	68	NH1	ARG	4	1.048	-5.233	0.460	1.00	0.00
ATOM	69	HH11	ARG	4	0.750	-5.424	1.395	1.00	0.00
ATOM	70	HH12	ARG	4	0.942	-5.927	-0.252	1.00	0.00
ATOM	71	NH2	ARG	4	1.965	-3.825	-1.052	1.00	0.00
ATOM	72	HH21	ARG	4	2.370	-2.938	-1.276	1.00	0.00
ATOM	73	HH22	ARG	4	1.859	-4.520	-1.764	1.00	0.00
ATOM	74	C	ARG	4	-0.156	0.835	-1.306	1.00	0.00
ATOM	75	O	ARG	4	0.292	-0.044	-2.015	1.00	0.00
ATOM	76	N	ARG	5	0.150	2.088	-1.507	1.00	0.00
ATOM	77	HN	ARG	5	-0.225	2.805	-0.970	1.00	0.00
ATOM	78	CA	ARG	5	1.062	2.546	-2.605	1.00	0.00
ATOM	79	HA	ARG	5	1.447	1.693	-3.157	1.00	0.00
ATOM	80	CB	ARG	5	0.167	3.349	-3.540	1.00	0.00
ATOM	81	HB1	ARG	5	0.684	3.496	-4.489	1.00	0.00
ATOM	82	HB2	ARG	5	-0.044	4.319	-3.089	1.00	0.00
ATOM	83	CG	ARG	5	-1.142	2.596	-3.784	1.00	0.00
ATOM	84	HG1	ARG	5	-1.832	3.235	-4.334	1.00	0.00
ATOM	85	HG2	ARG	5	-1.587	2.319	-2.828	1.00	0.00
ATOM	86	CD	ARG	5	-0.861	1.333	-4.602	1.00	0.00
ATOM	87	HD1	ARG	5	-1.790	0.801	-4.798	1.00	0.00
ATOM	88	HD2	ARG	5	-0.168	0.687	-4.058	1.00	0.00
ATOM	89	NE	ARG	5	-0.259	1.834	-5.868	1.00	0.00
ATOM	90	HE	ARG	5	0.592	1.404	-6.094	1.00	0.00
ATOM	91	CZ	ARG	5	-0.804	2.756	-6.613	1.00	0.00
ATOM	92	NH1	ARG	5	-2.099	2.919	-6.607	1.00	0.00
ATOM	93	HH11	ARG	5	-2.673	2.338	-6.031	1.00	0.00
ATOM	94	HH12	ARG	5	-2.516	3.626	-7.178	1.00	0.00
ATOM	95	NH2	ARG	5	-0.054	3.515	-7.365	1.00	0.00
ATOM	96	HH21	ARG	5	0.938	3.389	-7.370	1.00	0.00
ATOM	97	HH22	ARG	5	-0.472	4.221	-7.936	1.00	0.00
ATOM	98	C	ARG	5	2.235	3.432	-2.176	1.00	0.00
ATOM	99	O	ARG	5	3.149	3.598	-2.959	1.00	0.00
ATOM	100	N	PRO	6	2.225	4.009	-0.990	1.00	0.00
ATOM	101	CA	PRO	6	3.362	4.885	-0.604	1.00	0.00

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ATOM	102	HA	PRO	6	3.562	5.623	-1.380	1.00	0.00
ATOM	103	CB	PRO	6	2.877	5.579	0.665	1.00	0.00
ATOM	104	HB1	PRO	6	2.405	6.534	0.423	1.00	0.00
ATOM	105	HB2	PRO	6	3.704	5.730	1.362	1.00	0.00
ATOM	106	CG	PRO	6	1.867	4.642	1.236	1.00	0.00
ATOM	107	HG1	PRO	6	1.119	5.192	1.780	1.00	0.00
ATOM	108	HG2	PRO	6	2.357	3.932	1.887	1.00	0.00
ATOM	109	CD	PRO	6	1.228	3.922	0.080	1.00	0.00
ATOM	110	HD2	PRO	6	1.038	2.890	0.343	1.00	0.00
ATOM	111	HD1	PRO	6	0.316	4.415	-0.219	1.00	0.00
ATOM	112	C	PRO	6	4.587	4.040	-0.334	1.00	0.00
ATOM	113	O	PRO	6	5.195	4.120	0.714	1.00	0.00
ATOM	114	N	SRP	7	4.973	3.235	-1.287	1.00	0.00
ATOM	115	HN	SRP	7	4.501	3.180	-2.153	1.00	0.00
ATOM	116	CA	SRP	7	6.178	2.345	-1.198	1.00	0.00
ATOM	117	C	SRP	7	5.986	1.217	-0.187	1.00	0.00
ATOM	118	O	SRP	7	6.890	0.401	0.000	1.00	0.00
ATOM	119	CB	SRP	7	7.409	3.196	-0.806	1.00	0.00
ATOM	120	OG1	SRP	7	7.614	4.247	-1.816	1.00	0.00
ATOM	121	PG2	SRP	7	9.111	4.826	-1.692	1.00	0.00
ATOM	122	OG3	SRP	7	9.841	4.741	-3.126	1.00	0.00
ATOM	123	OG2	SRP	7	9.883	4.016	-0.692	1.00	0.00
ATOM	124	OG4	SRP	7	9.056	6.362	-1.210	1.00	0.00
ATOM	125	HA	SRP	7	6.354	1.890	-2.170	1.00	0.00
ATOM	126	HG3	SRP	7	10.770	4.312	-3.012	1.00	0.00
ATOM	127	HG4	SRP	7	8.124	6.751	-1.413	1.00	0.00
ATOM	128	HB1	SRP	7	8.290	2.553	-0.751	1.00	0.00
ATOM	129	HB2	SRP	7	7.240	3.659	0.163	1.00	0.00
ATOM	130	N	TYR	8	4.845	1.147	0.452	1.00	0.00
ATOM	131	HN	TYR	8	4.121	1.772	0.312	1.00	0.00
ATOM	132	CA	TYR	8	4.521	0.098	1.463	1.00	0.00
ATOM	133	HA	TYR	8	4.788	0.421	2.466	1.00	0.00
ATOM	134	CB	TYR	8	3.001	-0.061	1.371	1.00	0.00
ATOM	135	HB1	TYR	8	2.757	-1.115	1.255	1.00	0.00
ATOM	136	HB2	TYR	8	2.631	0.495	0.510	1.00	0.00
ATOM	137	CG	TYR	8	2.351	0.471	2.630	1.00	0.00
ATOM	138	CD1	TYR	8	2.895	0.164	3.884	1.00	0.00
ATOM	139	HD1	TYR	8	3.776	-0.453	3.953	1.00	0.00
ATOM	140	CD2	TYR	8	1.202	1.269	2.544	1.00	0.00
ATOM	141	HD2	TYR	8	0.776	1.504	1.579	1.00	0.00
ATOM	142	CE1	TYR	8	2.293	0.655	5.048	1.00	0.00
ATOM	143	HE1	TYR	8	2.713	0.417	6.014	1.00	0.00
ATOM	144	CE2	TYR	8	0.600	1.759	3.710	1.00	0.00
ATOM	145	HE2	TYR	8	-0.284	2.374	3.643	1.00	0.00
ATOM	146	CZ	TYR	8	1.146	1.452	4.961	1.00	0.00
ATOM	147	OH	TYR	8	0.553	1.936	6.110	1.00	0.00
ATOM	148	HH	TYR	8	1.222	2.407	6.613	1.00	0.00
ATOM	149	C	TYR	8	5.198	-1.217	1.126	1.00	0.00
ATOM	150	O	TYR	8	5.057	-1.728	0.033	1.00	0.00
ATOM	151	N	ARG	9	5.936	-1.788	2.046	1.00	0.00
ATOM	152	HN	ARG	9	6.065	-1.397	2.951	1.00	0.00
ATOM	153	CA	ARG	9	6.655	-3.095	1.832	1.00	0.00
ATOM	154	HA	ARG	9	5.958	-3.901	1.569	1.00	0.00
ATOM	155	CB	ARG	9	7.597	-2.847	0.639	1.00	0.00
ATOM	156	HB1	ARG	9	7.078	-2.256	-0.115	1.00	0.00
ATOM	157	HB2	ARG	9	7.886	-3.805	0.204	1.00	0.00
ATOM	158	CG	ARG	9	8.858	-2.097	1.089	1.00	0.00
ATOM	159	HG1	ARG	9	8.772	-1.825	2.139	1.00	0.00
ATOM	160	HG2	ARG	9	8.975	-1.193	0.489	1.00	0.00
ATOM	161	CD	ARG	9	10.085	-2.996	0.895	1.00	0.00

					72				
ATOM	162	HD1	ARG	9	10.070	-3.810	1.617	1.00	0.00
ATOM	163	HD2	ARG	9	10.998	-2.408	1.013	1.00	0.00
ATOM	164	NE	ARG	9	9.950	-3.518	-0.493	1.00	0.00
ATOM	165	HE	ARG	9	9.658	-4.453	-0.534	1.00	0.00
ATOM	166	CZ	ARG	9	10.193	-2.808	-1.561	1.00	0.00
ATOM	167	NH1	ARG	9	11.414	-2.436	-1.833	1.00	0.00
ATOM	168	HH11	ARG	9	12.163	-2.695	-1.223	1.00	0.00
ATOM	169	HH12	ARG	9	11.600	-1.892	-2.651	1.00	0.00
ATOM	170	NH2	ARG	9	9.215	-2.471	-2.357	1.00	0.00
ATOM	171	HH21	ARG	9	8.280	-2.756	-2.149	1.00	0.00
ATOM	172	HH22	ARG	9	9.402	-1.927	-3.175	1.00	0.00
ATOM	173	C	ARG	9	7.449	-3.492	3.057	1.00	0.00
ATOM	174	OT1	ARG	9	7.344	-2.801	4.057	1.00	0.00
ATOM	175	OT2	ARG	9	8.155	-4.485	2.985	1.00	0.00
END									

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

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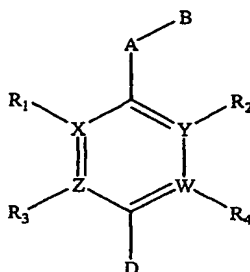
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Zhang, Z. H., Johnson, J. A., Chen, L., El-Sherif, N., Mochly-Rosen, D. & Boutjdir, M. *Circ. Res.* 80, 720-9 (1997)

WHAT IS CLAIMED IS:

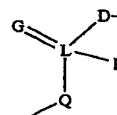
1. A compound having a general formula:

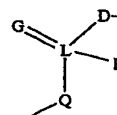


wherein:

X, Y, Z and W are each independently a carbon atom or a nitrogen atom;

A is alkyl or absent;



B is a negatively charged group having a formula , wherein L is selected from the group consisting of a phosphor atom, a sulfur atom, a silicon atom, a boron atom and a carbon atom; Q, G and D are each independently selected from the group consisting of oxygen and sulfur;; and E is selected from the group consisting of hydroxy, alkoxy, aryloxy, carbonyl, thiocarbonyl, O-carboxy, thiohydroxy, thioalkoxy and thioaryloxy or absent;

D is selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanyliinoalkyl, guanidino, guanidinoalkyl, amino, hydrazine, aminoalkyl and a hydrophobic moiety; and

R₁, R₂, R₃ and R₄ are each independently selected from the group consisting of hydrogen, a lone pair of electrons, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy,

thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and an ammonium ion,

or a pharmaceutically acceptable salt thereof,

provided that at least one of X, Y, Z and W is a nitrogen atom and/or at least one of R₁, R₂, R₃ and R₄ is a group containing at least one amino moiety, and with the proviso that the compound is not pyridoxal phosphate.

2. The compound of claim 1, being capable of inhibiting an activity of GSK-3.
3. The compound of claim 1, wherein A is alkyl.
4. The compound of claim 1, wherein L is a phosphor atom.
5. The compound of claim 4, wherein each of Q, G and D is oxygen.
6. The compound of claim 4, wherein E is hydroxy.
7. The compound of claim 1, wherein at least one of X, Y, Z and W is a nitrogen atom.
8. The compound of claim 7, wherein at least two of X, Y, Z and W are nitrogen atoms.
9. The compound of claim 8, wherein X and Y are each a nitrogen atom.
10. The compound of claim 8, wherein Z and W are each a nitrogen atom.

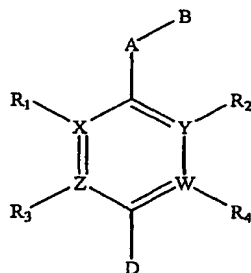
11. The compound of claim 1, wherein at least two of R_1 , R_2 , R_3 and R_4 are said groups containing at least one amino moiety.
12. The compound of claim 11, wherein each of R_1 and R_2 is said group containing at least one amino moiety.
13. The compound of claim 11, wherein each of R_3 and R_4 is said group containing at least one amino moiety.
14. The compound of claim 1, wherein D is a hydrophobic moiety.
15. The compound of claim 14, wherein said hydrophobic moiety is selected from the group consisting of a fatty acid residue, a saturated alkylene chain having between 4 and 30 carbon atoms, an unsaturated alkylene chain having between 4 and 30 carbon atoms, an aryl, a cycloalkyl and a hydrophobic peptide sequence.
16. The compound of claim 15, wherein said fatty acid is selected from the group consisting of myristic acid, lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid, linoleic acid and linolenic acid.
17. The compound of claim 5, wherein A is alkyl, each of X, Y, Z and W is a carbon atom, and at least one of R_3 and R_4 is said group containing at least one amino moiety.
18. The compound of claims 1-17, wherein said at least one amino moiety is selected from the group consisting of guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, guanyl, guanylinoalkyl, and any combination thereof.
19. The compound of claims 1-17, wherein said group containing at least one amino moiety comprises at least one positively charged group.
20. The compound of claim 19, wherein said at least one positively charged group comprises an ammonium ion.

21. The compound of claim 19, wherein said at least one positively charged group has a chemical structure derived from a side chain of a positively charged amino acid.

22. The compound of claim 21, wherein said positively charged amino acid is selected from the group consisting of arginine, lysine, histidine, proline and any derivative thereof.

23. The compound of claim 1, wherein each of X, Y and Z is a carbon atom and W is a nitrogen atom.

24. A compound having a general formula:

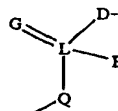


Formula I

wherein:

X, Y, Z and W are each independently a carbon atom or a nitrogen atom;

A is alkyl or absent;



B is a negatively charged group having a formula $\begin{array}{c} \text{G} \\ \diagup \\ \text{L} \\ \diagdown \\ \text{Q} \end{array} \text{D}^- \text{E}$, wherein L is selected from the group consisting of a phosphorus atom, a sulfur atom, a silicon atom, a boron atom and a carbon atom; Q, G and D are each independently selected from the group consisting of oxygen and sulfur;; and E is selected from the group consisting of

hydroxy, alkoxy, aryloxy, carbonyl, thiocarbonyl, O-carboxy, thiohydroxy, thioalkoxy and thioaryloxy or absent;

D is a hydrophobic moiety; and

R₁, R₂, R₃ and R₄ are each independently selected from the group consisting of hydrogen, a lone pair of electrons, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, hydrazine, aminoalkyl and an ammonium ion,

or a pharmaceutically acceptable salt thereof.

25. The compound of claim 24, being capable of inhibiting an activity of GSK-3.

26. The compound of claim 24, wherein said hydrophobic moiety is selected from the group consisting of a fatty acid residue, a saturated alkylene chain having between 4 and 30 carbon atoms, an unsaturated alkylene chain having between 4 and 30 carbon atoms, an aryl, a cycloalkyl and a hydrophobic peptide sequence.

27. The compound of claim 26, wherein said fatty acid is selected from the group consisting of myristic acid, lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid, linoleic acid and linolenic acid.

28. The compound of claim 24, wherein A is alkyl.

29. The compound of claim 24, wherein L is a phosphor atom.

30. The compound of claim 29, wherein each of Q, G and D is oxygen.

31. The compound of claim 29, wherein E is hydroxy.

32. The compound of claim 24, wherein at least one of X, Y, Z and W is a nitrogen atom.
33. The compound of claim 32, wherein at least two of X, Y, Z and W are nitrogen atoms.
34. The compound of claim 33, wherein X and Y are each a nitrogen atom.
35. The compound of claim 33, wherein Z and W are each a nitrogen atom.
36. The compound of claim 32, wherein W is a nitrogen atom.
37. The compound of claim 24, wherein at least one of R₁, R₂, R₃ and R₄ is a group containing at least one amino moiety.
38. The compound of claim 37, wherein at least two of R₁, R₂, R₃ and R₄ are said groups containing at least one amino moiety.
39. The compound of claim 38, wherein each of R₁ and R₂ is said group containing at least one amino moiety.
40. The compound of claim 38, wherein each of R₃ and R₄ is said group containing at least one amino moiety.
41. The compound of claim 30, wherein A is alkyl.
42. The compound of claim 41, wherein each of X, Y, Z and W is a carbon atom.
43. The compound of claim 42, wherein each of R₁, R₂, R₃ and R₄ is hydrogen.

44. The compound of claim 41, wherein each of X, Y and Z is a carbon atom and W is a nitrogen atom.

45. The compound of claim 42, wherein at least one of R₃ and R₄ is a group containing at least one amino moiety.

46. The compound of claims 37-40 and 45, wherein said at least one amino moiety is selected from the group consisting of guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, guanyl, guanyliinoalkyl, and any combination thereof.

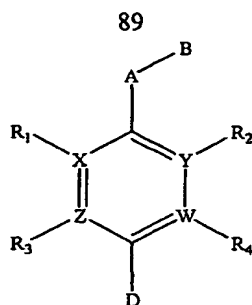
47. The compound of claims 37-40 and 45, wherein said group containing at least one amino moiety comprises at least one positively charged group.

48. The compound of claim 47, wherein said at least one positively charged group comprises an ammonium ion.

49. The compound of claim 47, wherein said at least one positively charged group has a chemical structure derived from a side chain of a positively charged amino acid.

50. The compound of claim 49, wherein said positively charged amino acid is selected from the group consisting of arginine, lysine, histidine, proline and any derivative thereof.

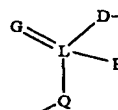
51. A pharmaceutical composition comprising, as an active ingredient, a compound having the general formula:



wherein:

X, Y, Z and W are each independently a carbon atom or a nitrogen atom;

A is alkyl or absent;



B is a negatively charged group having a formula $\begin{array}{c} \text{D}^- \\ | \\ \text{G} = \text{L} - \text{E} \\ | \\ \text{Q} \end{array}$, wherein L is selected from the group consisting of a phosphorus atom, a sulfur atom, a silicon atom, a boron atom and a carbon atom; Q, G and D are each independently selected from the group consisting of oxygen and sulfur;; and E is selected from the group consisting of hydroxy, alkoxy, aryloxy, carbonyl, thiocarbonyl, O-carboxy, thiohydroxy, thioalkoxy and thioaryloxy or absent;

D is selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and a hydrophobic moiety; and

R₁, R₂, R₃ and R₄ are each independently selected from the group consisting of hydrogen, a lone pair of electrons, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl,

guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, and an ammonium ion,

or a pharmaceutically acceptable salt thereof,

said compound being capable of inhibiting an activity of GSK-3,

and a pharmaceutically acceptable carrier.

52. The pharmaceutical composition of claim 51, wherein A is alkyl.

53. The pharmaceutical composition of claim 51, wherein L is a phosphor atom.

54. The pharmaceutical composition of claim 53, wherein each of Q, G and D is oxygen.

55. The pharmaceutical composition of claim 54, wherein E is hydroxy.

56. The pharmaceutical composition of claim 51, wherein at least one of X, Y, Z and W is a nitrogen atom.

57. The pharmaceutical composition of claim 56, wherein at least two of X, Y, Z and W are nitrogen atoms.

58. The pharmaceutical composition of claim 57, wherein X and Y are each a nitrogen atom.

59. The pharmaceutical composition of claim 57, wherein Z and W are each a nitrogen atom.

60. The pharmaceutical composition of claim 51, wherein D is a hydrophobic moiety.

61. The pharmaceutical composition of claim 60, wherein said hydrophobic moiety is selected from the group consisting of a fatty acid residue, a

saturated alkylene chain having between 4 and 30 carbon atoms, an unsaturated alkylene chain having between 4 and 30 carbon atoms, an aryl, a cycloalkyl and a hydrophobic peptide sequence.

62. The pharmaceutical composition of claim 61, wherein said fatty acid is selected from the group consisting of myristic acid, lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid, linoleic acid and linolenic acid.

63. The pharmaceutical composition of claim 54, wherein A is alkyl.

64. The pharmaceutical composition of claim 63, wherein each of X, Y, Z and W is a carbon atom.

65. The pharmaceutical composition of claim 64, wherein each of D, R₁, R₂, R₃ and R₄ is hydrogen.

66. The pharmaceutical composition of claim 64, wherein D is alkyl and each of R₁, R₂, R₃ and R₄ is hydrogen.

67. The pharmaceutical composition of claim 63, wherein each of X, Y and Z is a carbon atom and W is a nitrogen atom.

68. The pharmaceutical composition of claims 51-67, wherein at least one of R₁, R₂, R₃ and R₄ is a group containing at least one amino moiety.

69. The pharmaceutical composition of claim 68, wherein at least two of R₁, R₂, R₃ and R₄ are said groups containing at least one amino moiety.

70. The pharmaceutical composition of claim 69, wherein each of R₁ and R₂ is said group containing at least one amino acid moiety.

71. The pharmaceutical composition of claim 69, wherein each of R₃ and R₄ is said group containing at least one amino acid moiety.

72. The pharmaceutical composition of claims 68-71, wherein said at least one amino moiety is selected from the group consisting of guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, guanyl, guanylinoalkyl, and any combination thereof.

73. The pharmaceutical composition of claims 68-71, wherein said group containing at least one amino acid moiety comprises at least one positively charged group.

74. The pharmaceutical composition of claim 73, wherein said at least one positively charged group comprises an ammonium ion.

75. The pharmaceutical composition of claim 73, wherein said positively charged group has a chemical structure derived from a side chain of a positively charged amino acid.

76. The pharmaceutical composition of claim 75, wherein said positively charged amino acid is selected from the group consisting of arginine, lysine, histidine, proline and any derivative thereof.

77. The pharmaceutical composition of claim 51, packaged in a packaging material and identified in print, on or in said packaging material, for use in the treatment of a biological condition associated with GSK-3 activity.

78. The pharmaceutical composition of claim 77, wherein said biological condition is selected from the group consisting of obesity, non-insulin dependent diabetes mellitus, an insulin-dependent condition, an affective disorder, a neurodegenerative disease or disorder and a psychotic disease or disorder.

79. The pharmaceutical composition of claim 78, wherein said affective disorder is selected from the group consisting of a unipolar disorder and a bipolar disorder.

80. The pharmaceutical composition of claim 79, wherein said unipolar disorder is depression.

81. The pharmaceutical composition of claim 79, wherein said bipolar disorder is manic depression.

82. The pharmaceutical composition of claim 78, wherein said neurodegenerative disorder results from an event selected from the group consisting of cerebral ischemia, stroke, traumatic brain injury and bacterial infection.

83. The pharmaceutical composition of claim 78, wherein said neurodegenerative disorder is a chronic neurodegenerative disorder.

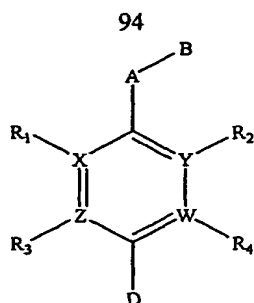
84. The pharmaceutical composition of claim 83, wherein said chronic neurodegenerative disorder results from a disease selected from the group consisting of Alzheimer's disease, Huntington's disease, Parkinson's disease, AIDS associated dementia, amyotrophic lateral sclerosis (AML) and multiple sclerosis.

85. The pharmaceutical composition of claim 51, further comprising at least one additional active ingredient that is capable of altering an activity of GSK-3.

86. The pharmaceutical composition of claim 85, wherein said additional active ingredient is capable of inhibiting an activity of GSK-3.

87. The pharmaceutical composition of claim 85, wherein said additional active ingredient is capable of downregulating an expression of GSK-3.

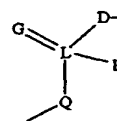
88. A method of inhibiting an activity of GSK-3, the method comprising contacting cells expressing GSK-3 with an inhibitory effective amount of a compound having a general formula:

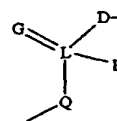


wherein:

X, Y, Z and W are each independently a carbon atom or a nitrogen atom;

A is alkyl or absent;



B is a negatively charged group having a formula , wherein L is selected from the group consisting of a phosphor atom, a sulfur atom, a silicon atom, a boron atom and a carbon atom; Q, G and D are each independently selected from the group consisting of oxygen and sulfur;; and E is selected from the group consisting of hydroxy, alkoxy, aryloxy, carbonyl, thiocarbonyl, O-carboxy, thiohydroxy, thioalkoxy and thioaryloxy or absent;

D is selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and a hydrophobic moiety; and

R₁, R₂, R₃ and R₄ are each independently selected from the group consisting of hydrogen, a lone pair of electrons, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl,

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guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, and an ammonium ion,

or a pharmaceutically acceptable salt thereof,

said compound being capable of inhibiting an activity of GSK-3.

89. The method of claim 88, wherein A is alkyl.
90. The method of claim 88, wherein L is a phosphor atom.
91. The method of claim 90, wherein each of Q, G and D is oxygen.
92. The method of claim 91, wherein E is hydroxy.
93. The method of claim 88, wherein at least one of X, Y, Z and W is a nitrogen atom.
94. The method of claim 93, wherein at least two of X, Y, Z and W are nitrogen atoms.
95. The method of claim 94, wherein X and Y are each a nitrogen atom.
96. The method of claim 94, wherein Z and W are each a nitrogen atom.
97. The method of claim 88, wherein D is a hydrophobic moiety.
98. The method of claim 97, wherein said hydrophobic moiety is selected from the group consisting of a fatty acid residue, a saturated alkylene chain having between 4 and 30 carbon atoms, an unsaturated alkylene chain having between 4 and 40 carbon atoms, an aryl, a cycloalkyl and a hydrophobic peptide sequence.
99. The method of claim 98, wherein said fatty acid is selected from the group consisting of myristic acid, lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid, linoleic acid and linolenic acid.

100. The method of claim 91, wherein A is alkyl.
101. The method of claim 100, wherein each of X, Y, Z and W is a carbon atom.
102. The method of claim 101, wherein each of D, R₁, R₂, R₃ and R₄ is hydrogen.
103. The method of claim 101, wherein D is alkyl and each of R₁, R₂, R₃ and R₄ is hydrogen.
104. The method of claim 100, wherein each of X, Y and Z is a carbon atom and W is a nitrogen atom.
105. The method of claims 88-104, wherein at least one of R₁, R₂, R₃ and R₄ is a group containing at least one amino moiety.
106. The method of claim 105, wherein at least two of R₁, R₂, R₃ and R₄ are said groups containing at least one amino moiety.
107. The method of claim 106, wherein each of R₁ and R₂ is said group containing at least one amino acid moiety.
108. The method of claim 106, wherein each of R₃ and R₄ is said group containing at least one amino acid moiety.
109. The method of claims 105-108, wherein said at least one amino moiety is selected from the group consisting of guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, guanyl, guanylinoalkyl, and any combination thereof.
110. The method of claims 105-108, wherein said group containing at least one amino acid moiety comprises at least one positively charged group.

111. The method of claim 110, wherein said at least one positively charged group comprises an ammonium ion.

112. The method of claim 110, wherein said positively charged group has a chemical structure derived from a side chain of a positively charged amino acid.

113. The method of claim 112, wherein said positively charged amino acid is selected from the group consisting of arginine, lysine, histidine, proline and any derivative thereof.

114. The method of claim 88, wherein said activity is a phosphorylation activity and/or an autophosphorylation activity.

115. The method of claim 88, wherein said contacting is effected *in vitro*.

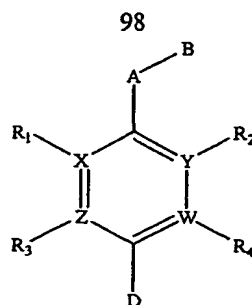
116. The method of claim 88, wherein said contacting is effected *in vivo*.

117. The method of claim 88, further comprising contacting said cells with at least one an additional active ingredient, said additional active ingredient being capable of altering an activity of GSK-3.

118. The method of claim 117, wherein said additional active ingredient is capable of inhibiting an activity of GSK-3.

119. The method of claim 117, wherein said additional active ingredient is capable of downregulating an expression of GSK-3.

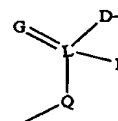
120. A method of potentiating insulin signaling, the method comprising contacting insulin responsive cells with an effective amount of a compound having a general formula:

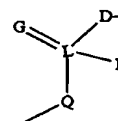


wherein:

X, Y, Z and W are each independently a carbon atom or a nitrogen atom;

A is alkyl or absent;



B is a negatively charged group having a formula , wherein L is selected from the group consisting of a phosphor atom, a sulfur atom, a silicon atom, a boron atom and a carbon atom; Q, G and D are each independently selected from the group consisting of oxygen and sulfur;; and E is selected from the group consisting of hydroxy, alkoxy, aryloxy, carbonyl, thiocarbonyl, O-carboxy, thiohydroxy, thioalkoxy and thioaryloxy or absent;

D is selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and a hydrophobic moiety; and

R₁, R₂, R₃ and R₄ are each independently selected from the group consisting of hydrogen, a lone pair of electrons, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl,

guanylinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, and an ammonium ion,

or a pharmaceutically acceptable salt thereof,

said compound being capable of inhibiting an activity of GSK-3.

121. The method of claim 120, wherein A is alkyl.

122. The method of claim 120, wherein L is a phosphor atom.

123. The method of claim 122, wherein each of Q, G and D is oxygen.

124. The method of claim 122, wherein E is hydroxy.

125. The method of claim 120, wherein at least one of X, Y, Z and W is a nitrogen atom.

126. The method of claim 125, wherein at least two of X, Y, Z and W are nitrogen atoms.

127. The method of claim 126, wherein X and Y are each a nitrogen atom.

128. The method of claim 126, wherein Z and W are each a nitrogen atom.

129. The method of claim 120, wherein D is a hydrophobic moiety.

130. The method of claim 129, wherein said hydrophobic moiety is selected from the group consisting of a fatty acid residue, a saturated alkylene chain having between 4 and 30 carbon atoms, an unsaturated alkylene chain having between 4 and 30 carbon atoms, an aryl, a cycloalkyl and a hydrophobic peptide sequence.

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131. The method of claim 130, wherein said fatty acid is selected from the group consisting of myristic acid, lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid, linoleic acid and linolenic acid.

132. The method of claim 123, wherein A is alkyl.

133. The method of claim 132, wherein each of X, Y, Z and W is a carbon atom.

134. The method of claim 133, wherein each of D, R₁, R₂, R₃ and R₄ is hydrogen.

135. The method of claim 133, wherein D is alkyl and each of R₁, R₂, R₃ and R₄ is hydrogen.

136. The method of claim 132, wherein each of X, Y and Z is a carbon atom and W is a nitrogen atom.

137. The method of claims 120-136, wherein at least one of R₁, R₂, R₃ and R₄ is a group containing at least one amino moiety.

138. The method of claim 137, wherein at least two of R₁, R₂, R₃ and R₄ are said groups containing at least one amino moiety.

139. The method of claim 138, wherein each of R₁ and R₂ is said group containing at least one amino acid moiety.

140. The method of claim 138, wherein each of R₃ and R₄ is said group containing at least one amino acid moiety.

141. The method of claims 137-140, wherein said at least one amino moiety is selected from the group consisting of guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, guanyl, guanylinoalkyl, and any combination thereof.

142. The method of claims 137-140, wherein said group containing at least one amino acid moiety comprises at least one positively charged group.

143. The method of claim 142, wherein said at least one positively charged group comprises an ammonium ion.

144. The method of claim 142, wherein said positively charged group has a chemical structure derived from a side chain of a positively charged amino acid.

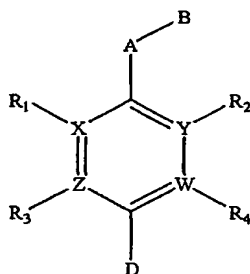
145. The method of claim 144, wherein said positively charged amino acid is selected from the group consisting of arginine, lysine, histidine, proline and any derivative thereof.

146. The method of claim 120, further comprising contacting said cells with insulin.

147. The method of claim 120, wherein said contacting is effected *in vitro*.

148. The method of claim 120, wherein said contacting is effected *in vivo*.

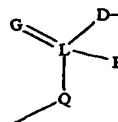
149. A method of treating a biological condition associated with an activity of GSK-3, the method comprising administering to a subject in need thereof a therapeutically effective amount of a compound having a general formula:

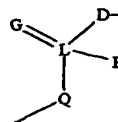


wherein:

X, Y, Z and W are each independently a carbon atom or a nitrogen atom;

A is alkyl or absent;



B is a negatively charged group having a formula , wherein L is selected from the group consisting of a phosphor atom, a sulfur atom, a silicon atom, a boron atom and a carbon atom; Q, G and D are each independently selected from the group consisting of oxygen and sulfur;; and E is selected from the group consisting of hydroxy, alkoxy, aryloxy, carbonyl, thiocarbonyl, O-carboxy, thiohydroxy, thioalkoxy and thioaryloxy or absent;

D is selected from the group consisting of hydrogen, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine and a hydrophobic moiety; and

R₁, R₂, R₃ and R₄ are each independently selected from the group consisting of hydrogen, a lone pair of electrons, alkyl, trihaloalkyl, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azo, sulfonamide, carbonyl, ketoester, thiocarbonyl, ester, ether, thioether, thiocarbamate, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, trihalomethanesulfonamido, guanyl, guanyliinoalkyl, guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, and an ammonium ion,

or a pharmaceutically acceptable salt thereof,

said compound being capable of inhibiting an activity of GSK-3.

150. The method of claim 149, wherein A is alkyl.

151. The method of claim 149, wherein L is a phosphor atom.
152. The method of claim 151, wherein each of Q, G and D is oxygen.
153. The method of claim 152, wherein E is hydroxy.
154. The method of claim 149, wherein at least one of X, Y, Z and W is a nitrogen atom.
155. The method of claim 154, wherein at least two of X, Y, Z and W are nitrogen atoms.
156. The method of claim 155, wherein X and Y are each a nitrogen atom.
157. The method of claim 155, wherein Z and W are each a nitrogen atom.
158. The method of claim 149, wherein D is a hydrophobic moiety.
159. The method of claim 158, wherein said hydrophobic moiety is selected from the group consisting of a fatty acid residue, a saturated alkylene chain having between 4 and 30 carbon atoms, an unsaturated alkylene chain having between 4 and 30 carbon atoms, an aryl, a cycloalkyl and a hydrophobic peptide sequence.
160. The method of claim 159, wherein said fatty acid is selected from the group consisting of myristic acid, lauric acid, palmitic acid, stearic acid, oleic acid, arachidonic acid, linoleic acid and linolenic acid.
161. The method of claim 152, wherein A is alkyl.
162. The method of claim 161, wherein each of X, Y, Z and W is a carbon atom.

163. The method of claim 162, wherein each of D, R₁, R₂, R₃ and R₄ is hydrogen.

164. The method of claim 162, wherein D is alkyl and each of R₁, R₂, R₃ and R₄ is hydrogen.

165. The method of claim 161, wherein each of X, Y and Z is a carbon atom and W is a nitrogen atom.

166. The method of claims 149-165, wherein at least one of R₁, R₂, R₃ and R₄ is a group containing at least one amino moiety.

167. The method of claim 166, wherein at least two of R₁, R₂, R₃ and R₄ are said groups containing at least one amino moiety.

168. The method of claim 167, wherein each of R₁ and R₂ is said group containing at least one amino acid moiety.

169. The method of claim 167, wherein each of R₃ and R₄ is said group containing at least one amino acid moiety.

170. The method of claims 166-169, wherein said at least one amino moiety is selected from the group consisting of guanidino, guanidinoalkyl, amino, aminoalkyl, hydrazine, guanyl, guanylinoalkyl, and any combination thereof.

171. The method of claims 166-169, wherein said group containing at least one amino acid moiety comprises at least one positively charged group.

172. The method of claim 171, wherein said at least one positively charged group comprises an ammonium ion.

173. The method of claim 171, wherein said positively charged group has a chemical structure derived from a side chain of a positively charged amino acid.

174. The method of claim 173, wherein said positively charged amino acid is selected from the group consisting of arginine, lysine, histidine, proline and any derivative thereof.

175. The method of claim 149, wherein said biological condition is selected from the group consisting of obesity, non-insulin dependent diabetes mellitus, an insulin-dependent condition, an affective disorder, a neurodegenerative disease or disorder and a psychotic disease or disorder.

176. The method of claim 175, wherein said affective disorder is selected from the group consisting of a unipolar disorder and a bipolar disorder.

177. The method of claim 176, wherein said unipolar disorder is depression.

178. The method of claim 176, wherein said bipolar disorder is manic depression.

179. The method of claim 175, wherein said neurodegenerative disorder results from an event selected from the group consisting of cerebral ischemia, stroke, traumatic brain injury and bacterial infection.

180. The method of claim 175, wherein said neurodegenerative disorder is a chronic neurodegenerative disorder.

181. The method of claim 180, wherein said chronic neurodegenerative disorder results from a disease selected from the group consisting of Alzheimer's disease, Huntington's disease, Parkinson's disease, AIDS associated dementia, amyotrophic lateral sclerosis (AML) and multiple sclerosis.

182. The method of claim 175, wherein said psychotic disorder is schizophrenia.

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183. The method of claim 149, further comprising co-administering to said subject at least one additional active ingredient, said at least one additional active ingredient being capable of altering an activity of GSK-3.

184. The method of claim 183, wherein said additional active ingredient is capable of inhibiting an activity of GSK-3.

185. The method of claim 183, wherein said additional active ingredient is capable of downregulating an expression of GSK-3.

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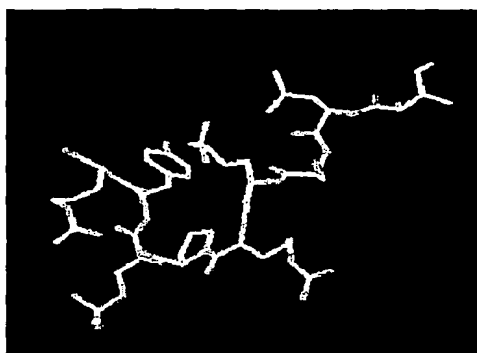


Fig. 1a

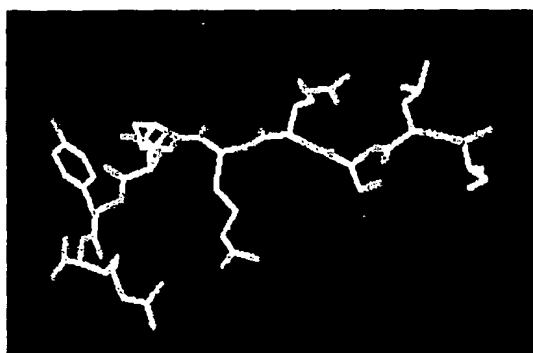


Fig. 1b

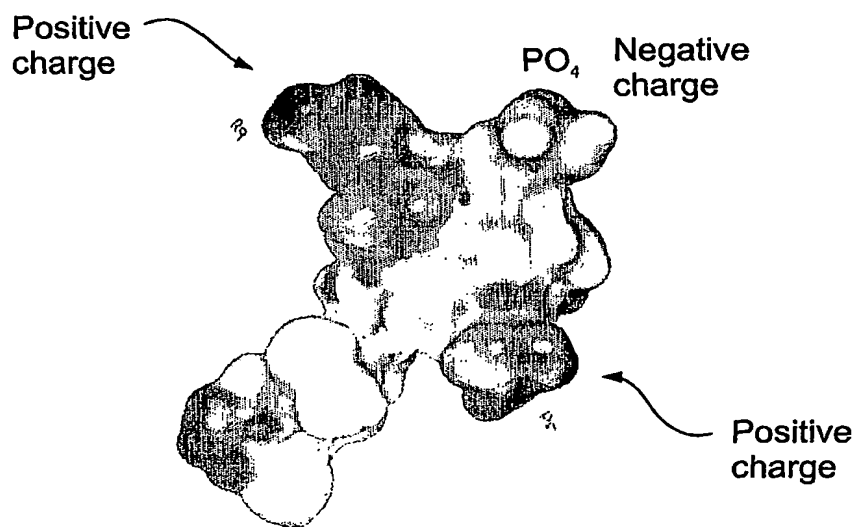
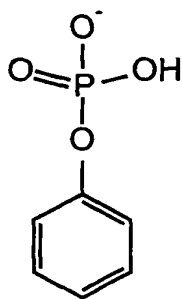
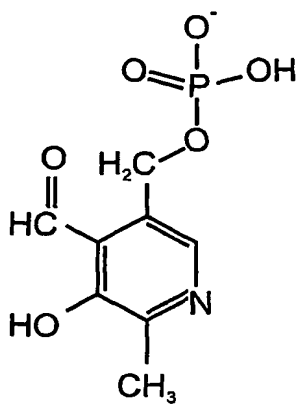


Fig. 2

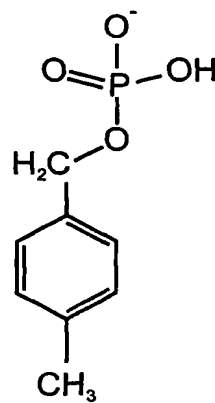
2/24



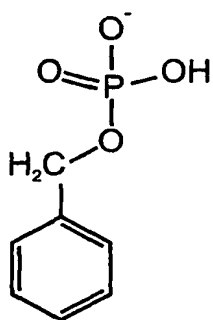
Phenyl Phosphate



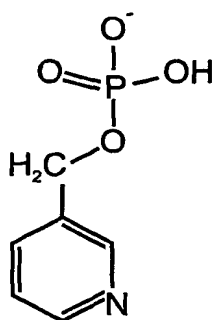
Pyridoxal Phosphate (P-5-P)



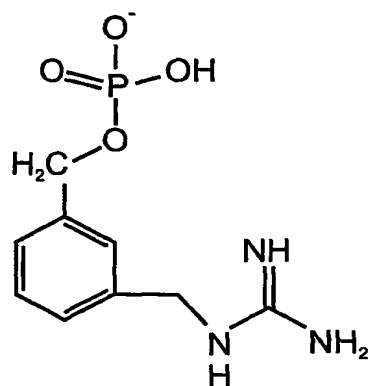
GS-1



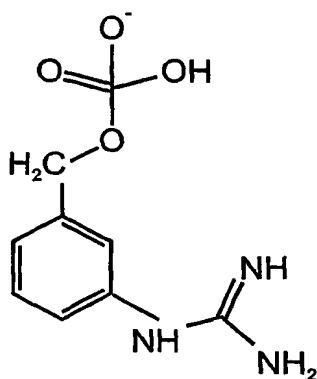
GS-2



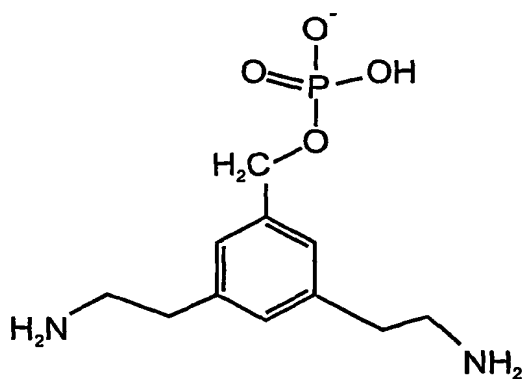
GS-3



GS-4



GS-5



GS-21

Fig. 3

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Current Data Parameters
NAME SMA-O-144-1
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20040208
Time 15.37
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 35.9
DW 48.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 10.00 usec
PL1 -2.00 dB
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300146 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

AMRI 21 Corporate circle
location; 9

4.477
4.488
4.500
4.511
4.521
4.531
4.541
4.551

7.136

NAME SLED
DATE 02/08/04
NB# SMA-O-144(1)

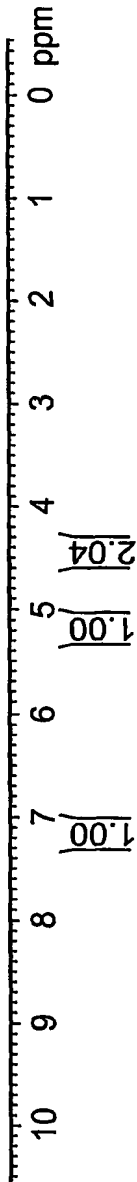
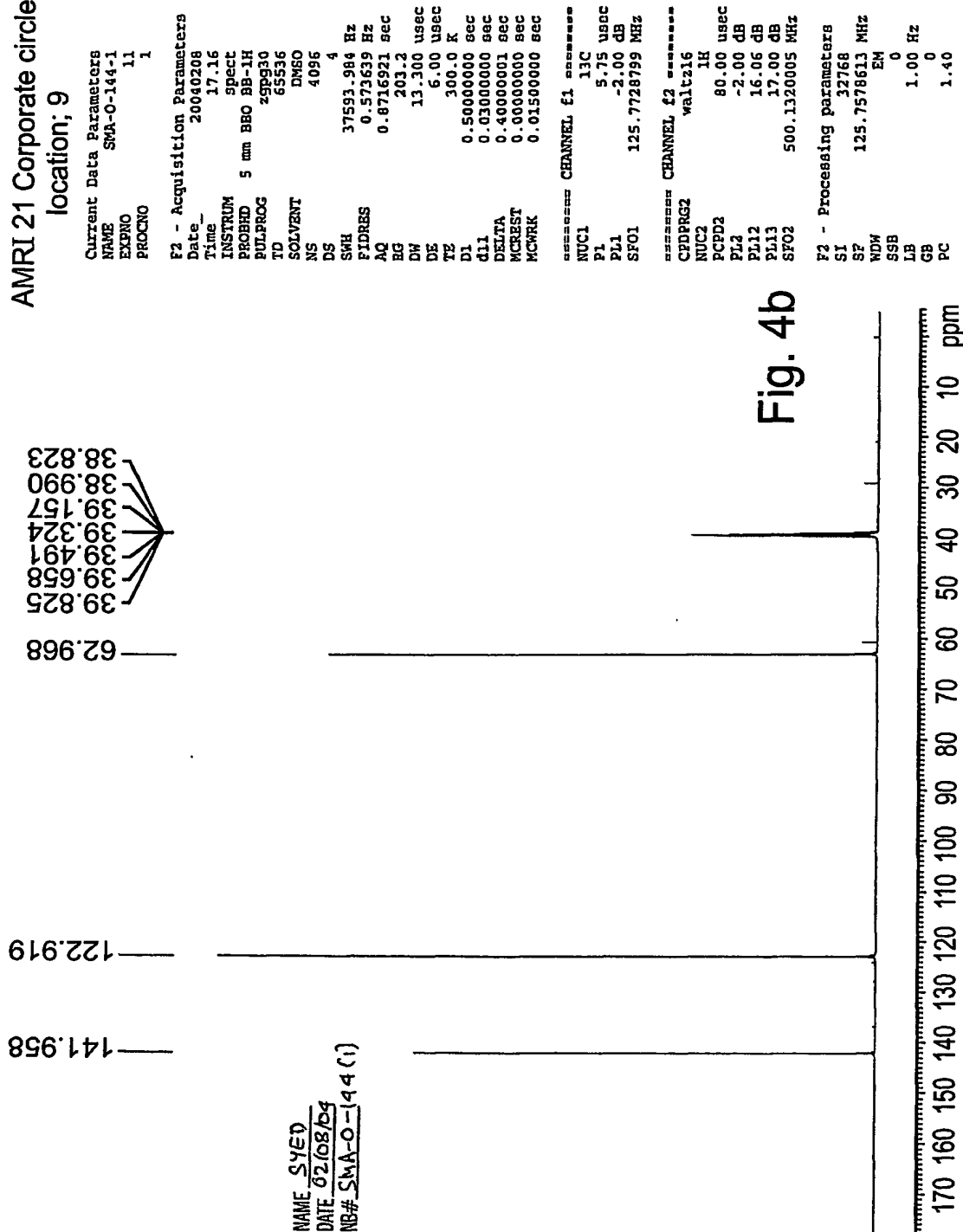


Fig. 4a

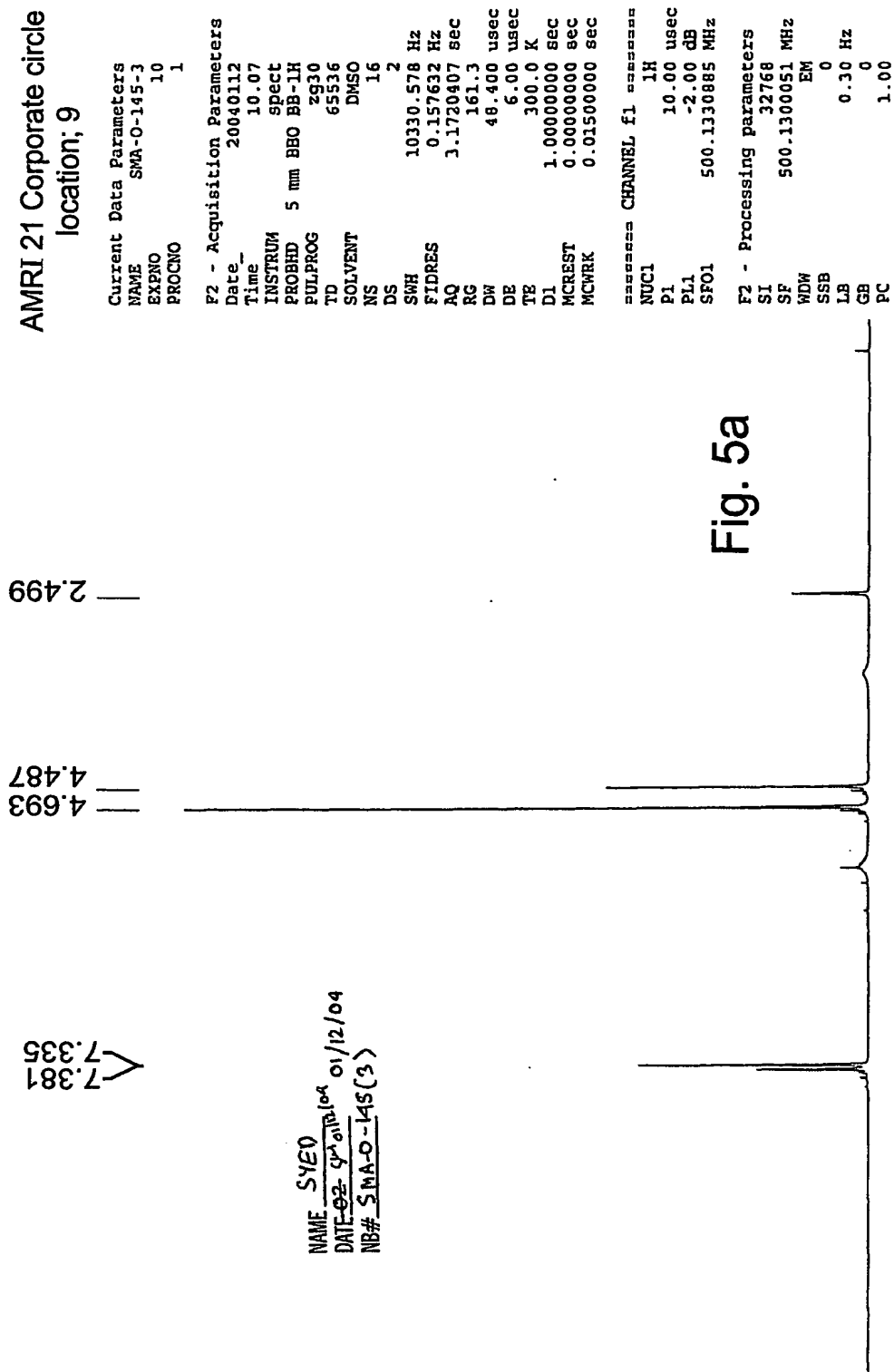
AMRI 21 Corporate circle
location; 9

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AMRI 21 Corporate circle
location; 9

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AMRI 21 Corporate circle
location; 9

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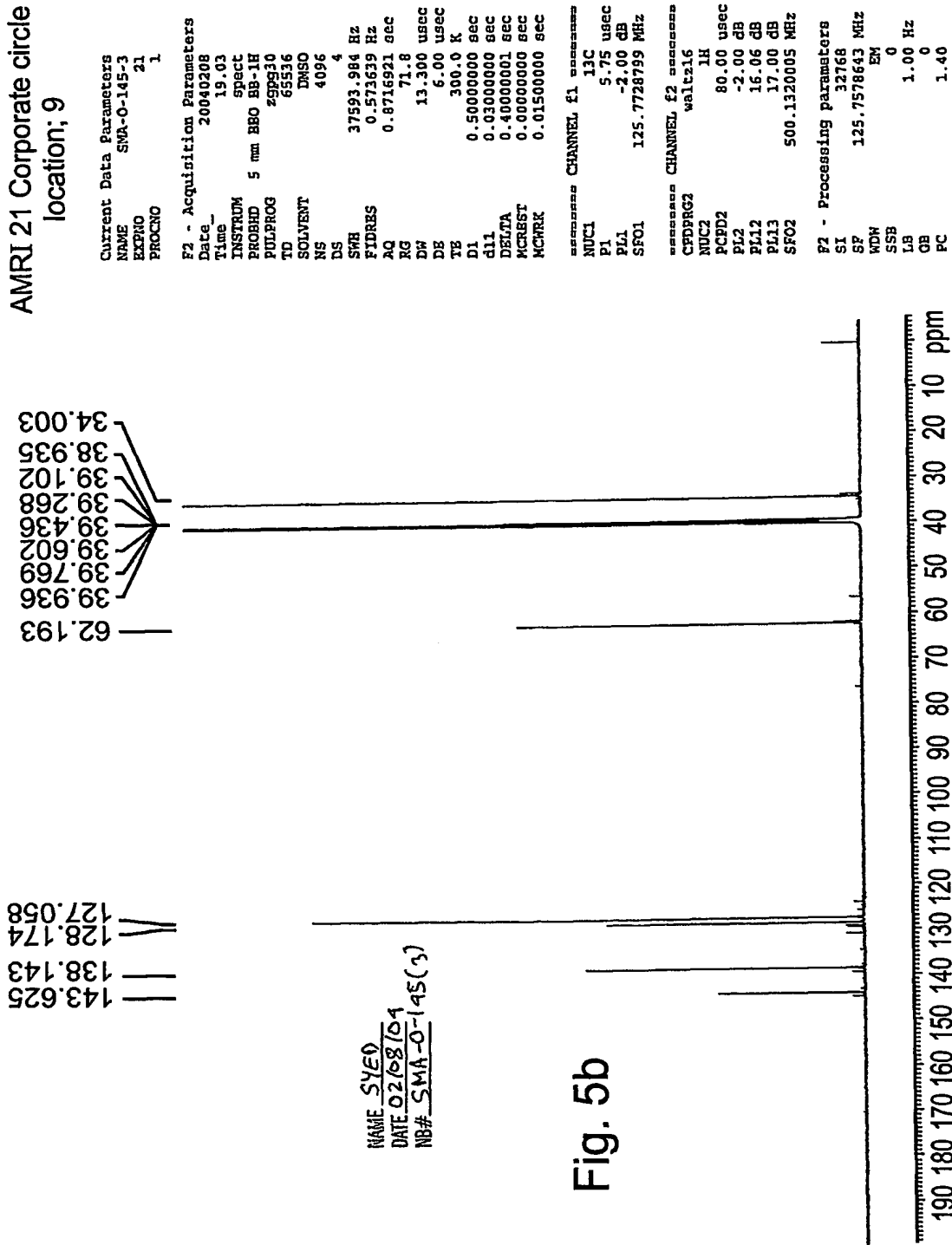


Fig. 5b

AMRI 21 Corporate circle
location; 9

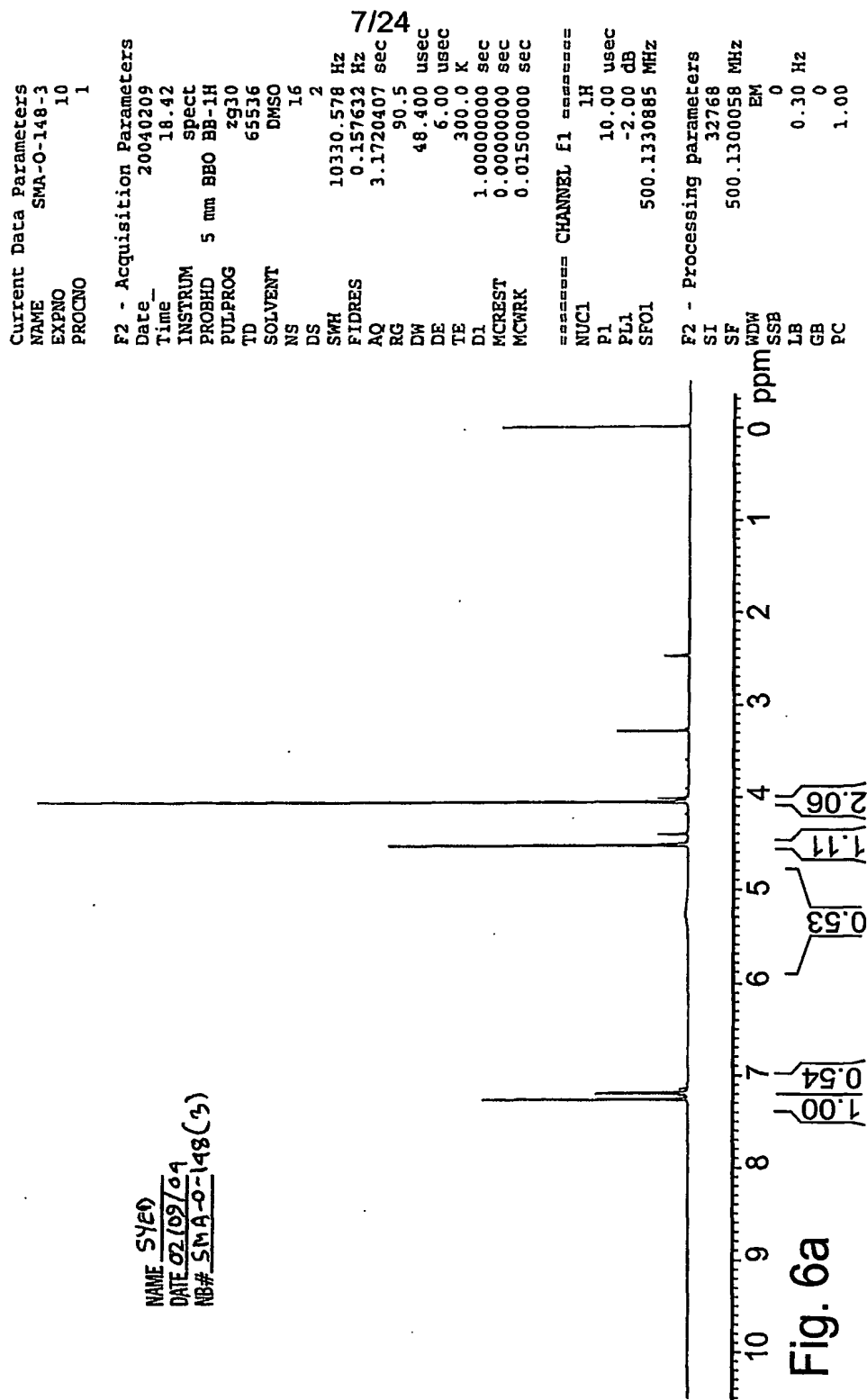
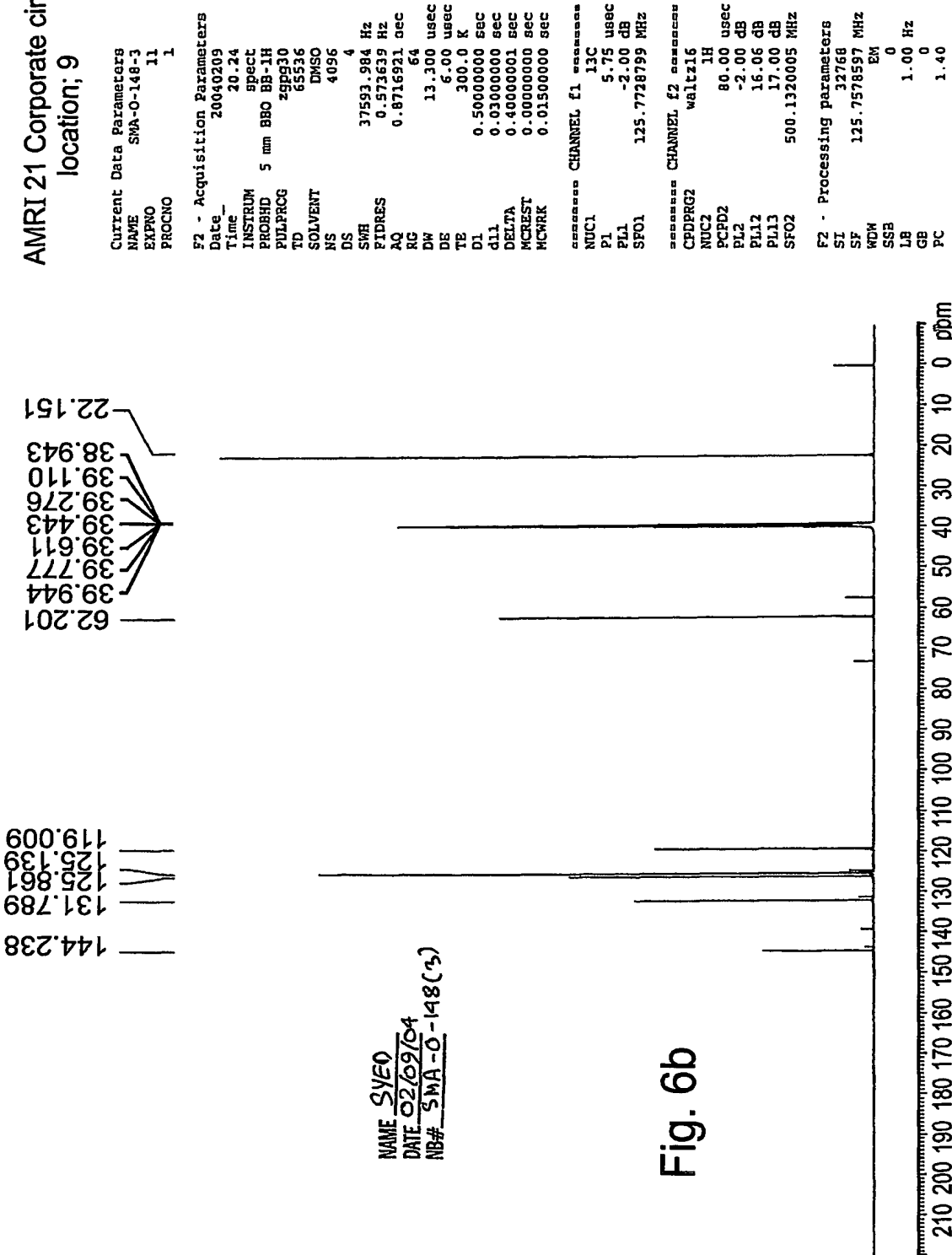


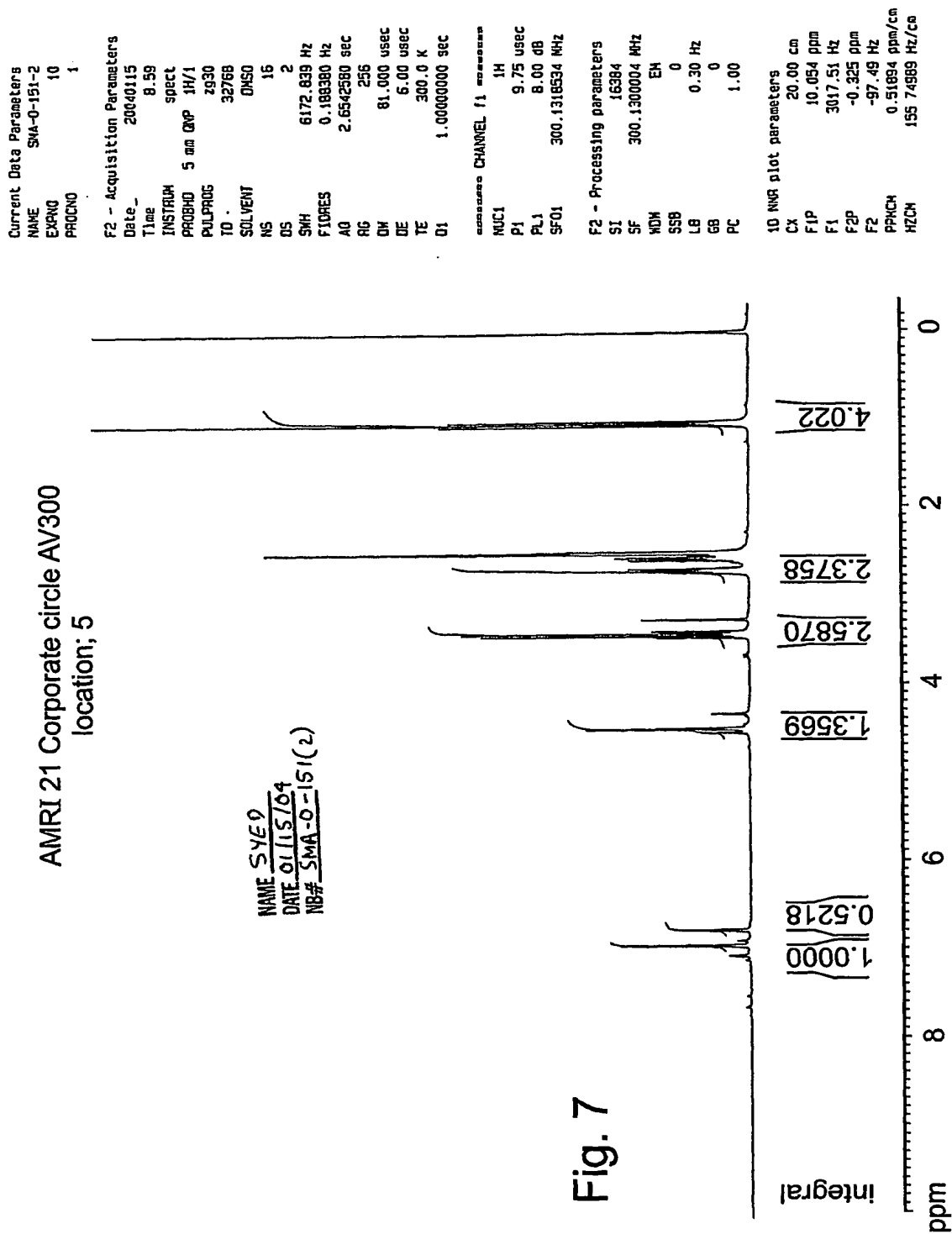
Fig. 6a

AMRI 21 Corporate circle
location; 9

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10/24

Current Data Parameters
NAME ams-f-32-1
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20040224
Time 8.02
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 2
SWH 6172.839 Hz
FIDRES 0.188380 Hz
AQ 2.6342980 sec
RG 287.4
DN 81.000 usec
DE 6.00 usec
TE 300.0 K
O1 1.00000000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 9.75 usec
PL1 8.00 dB
SF01 300.1318534 MHz

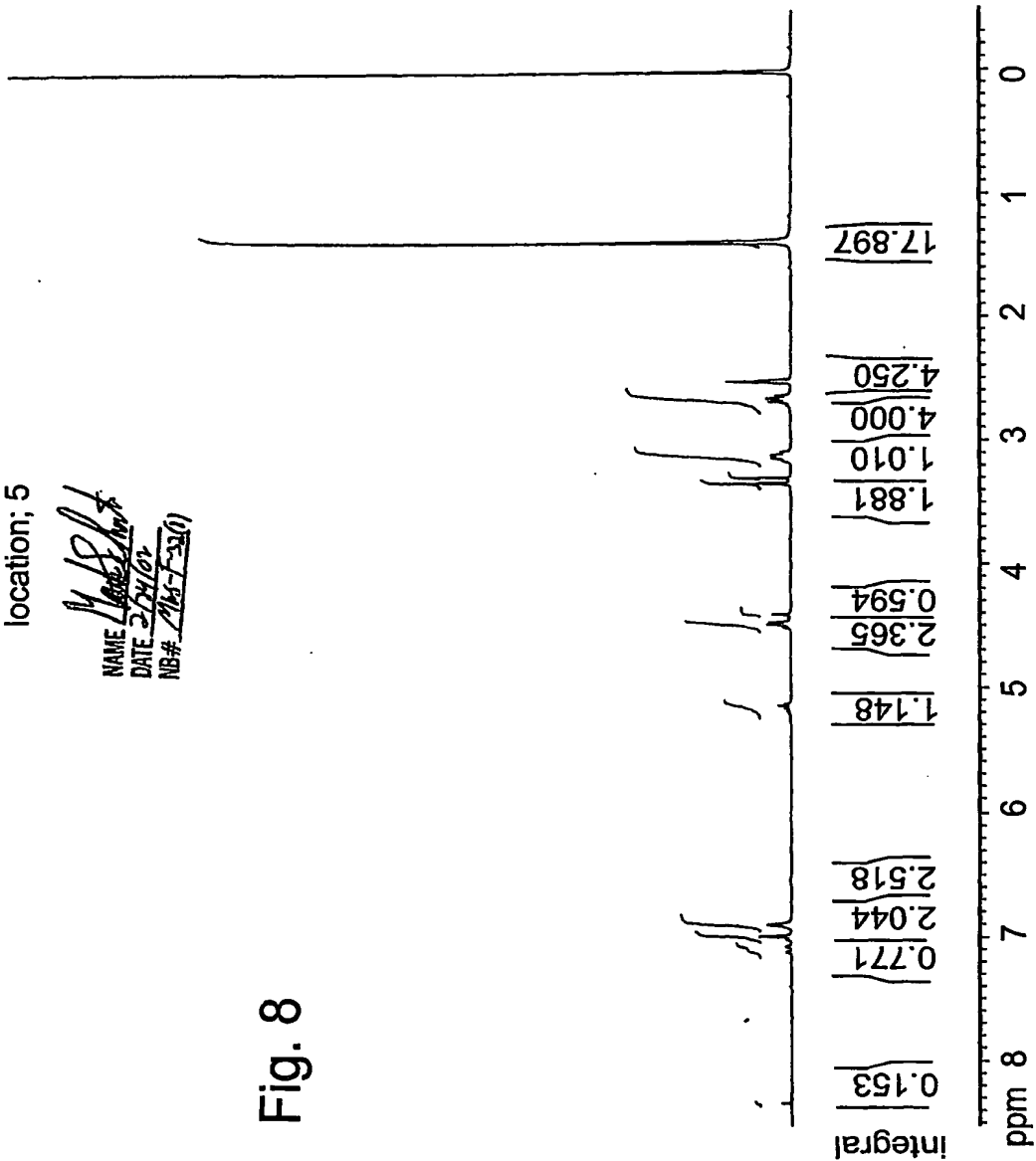
F2 - Processing parameters
SI 16384
SF 300.1314271 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

10 NMR plot parameters
CX 20.00 cm
F1P 8.500 ppm
F1 2551.12 Hz
F2P -0.500 ppm
F2 -150.07 Hz
PPHCH 0.45000 ppm/cm
HZCH 135.05914 Hz/cm

AMRI 21 Corporate circle AV300
location; 5

NAME ams-f-32-1
DATE 2/24/04
NB# 1761-32(1)

Fig. 8



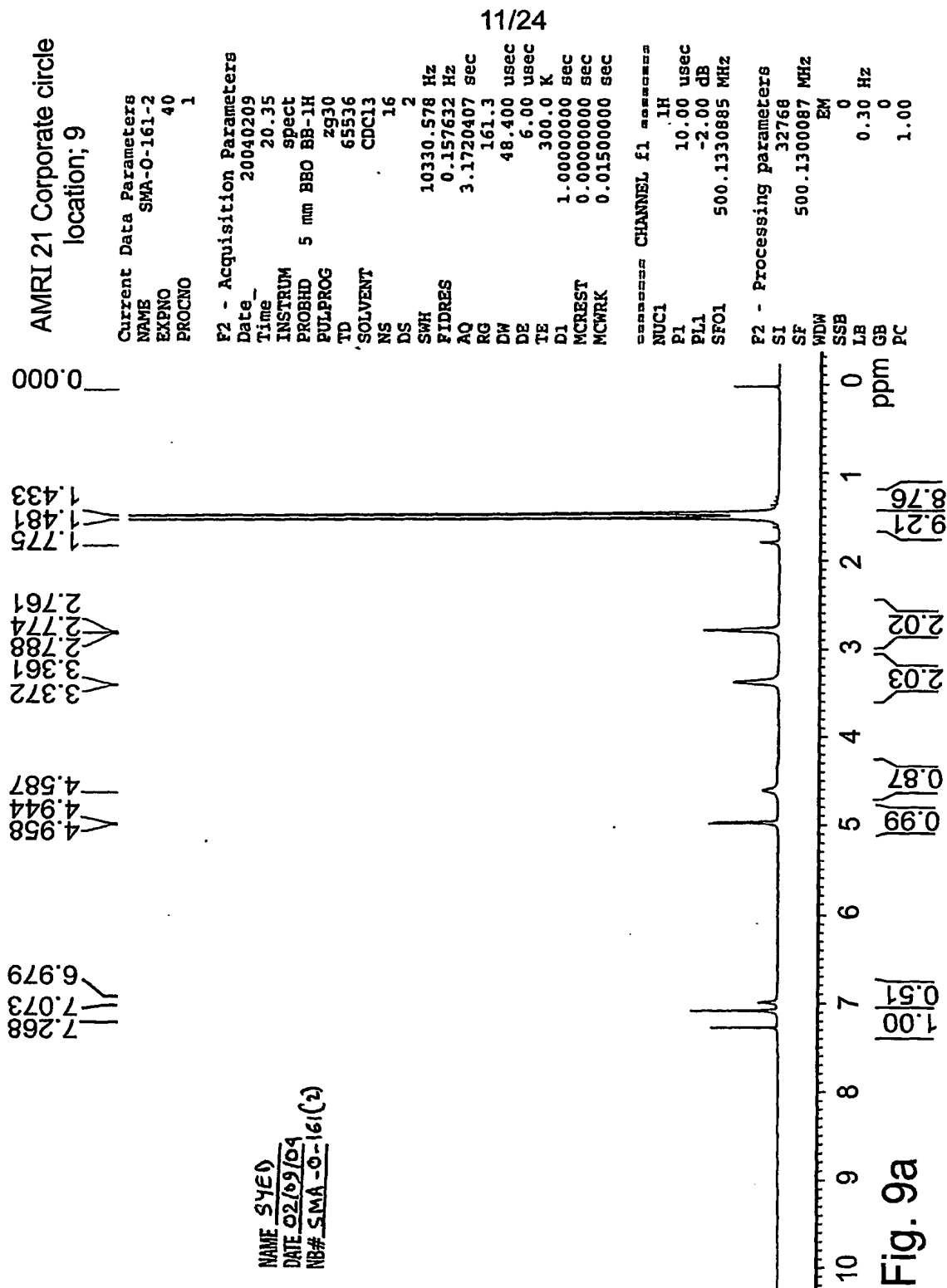


Fig. 9a

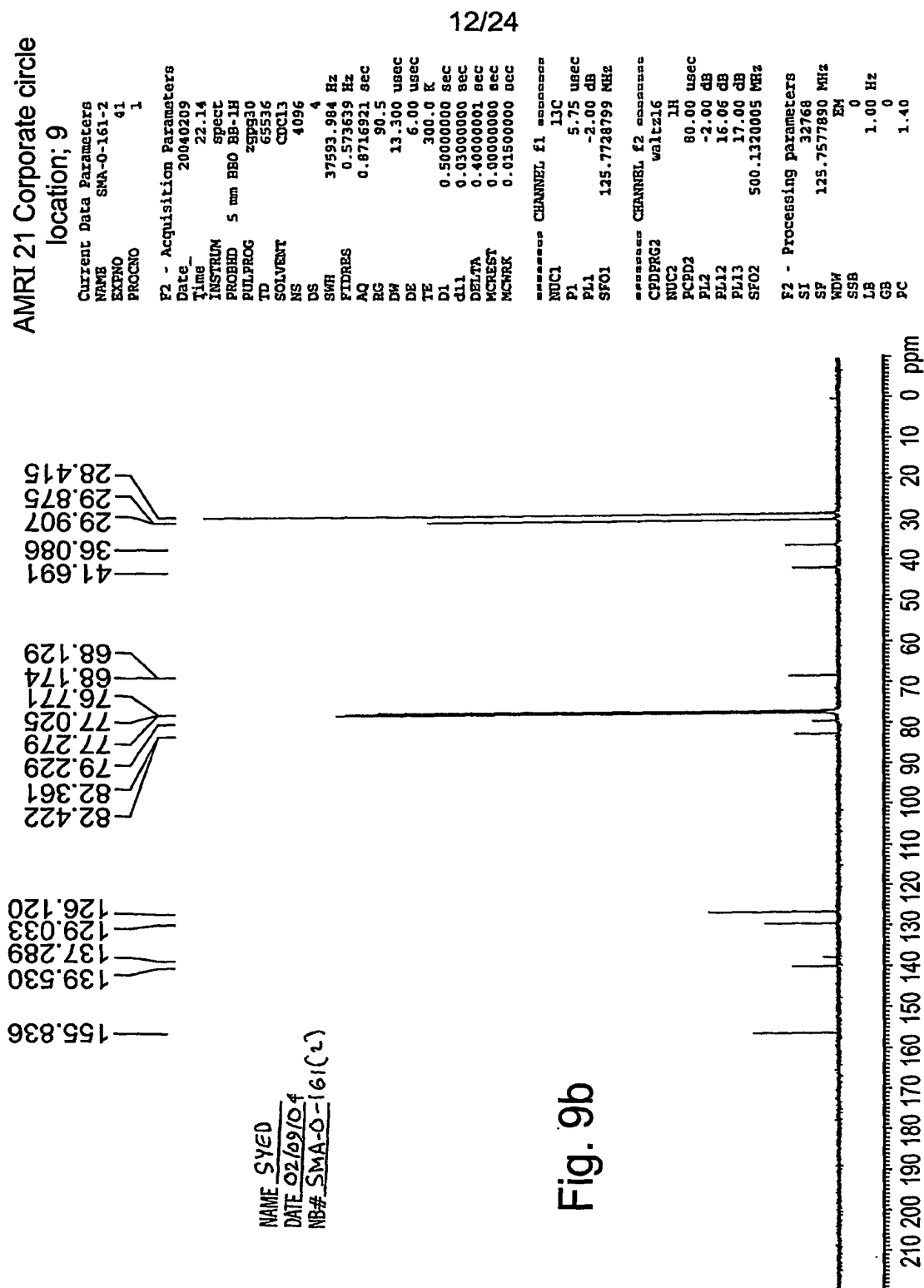
AMRI 21 Corporate circle
location; 9

Fig. 9b

NAME SYED
 DATE 02/09/04
 NB# SMA-O-161(2)

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Current Data Parameters
NAME SMA-O-161-2
EXPNO 42
PROCNO 1

P2 - Acquisition Parameters
Date_ 20040209
Time 22.19
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT CMC13
NS 16
DS 4
SWH 80645.164 Hz
FIDRES 1.230548 Hz
AQ 0.4063794 sec
RG 9195.2
DW 6.200 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.8959598 sec
MCHEST 0.00000000 sec
MCMRK 0.01500000 sec

===== CHANNEL f1 =====
NUC1 31P
P1 9.75 usec
PL1 0.00 dB
SFO1 202.4461871 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 2.00 dB
PL12 16.06 dB
PL13 17.00 dB
SFO2 500.1320005 MHz

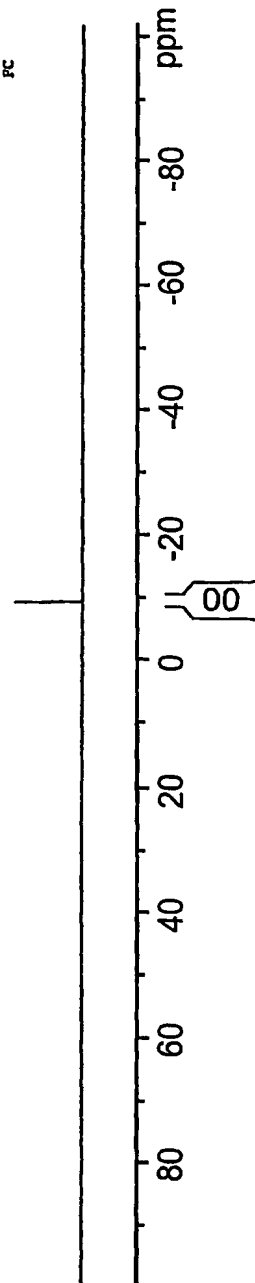
P2 - Processing parameters
SI 32768
SF 202.4563100 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

AMRI 21 Corporate circle
location; 9

69'6

NAME SYE0
DATE 02/02/04
NB# SMA-O-161(2)

Fig. 9c



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AMRI 21 Corporate circle

location; 9

Current Data Parameters
NAME SMA-O-189-1
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20040303
Time 18.41
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1720407 sec
RG 287.4
DW 48.400 usec
DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
MCREST 0.00000000 sec
MCWIRK 0.01500000 sec

===== CHANNEL f1 =====
NUC1 1H
P1 10.00 usec
PL1 -2.00 dB
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300128 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

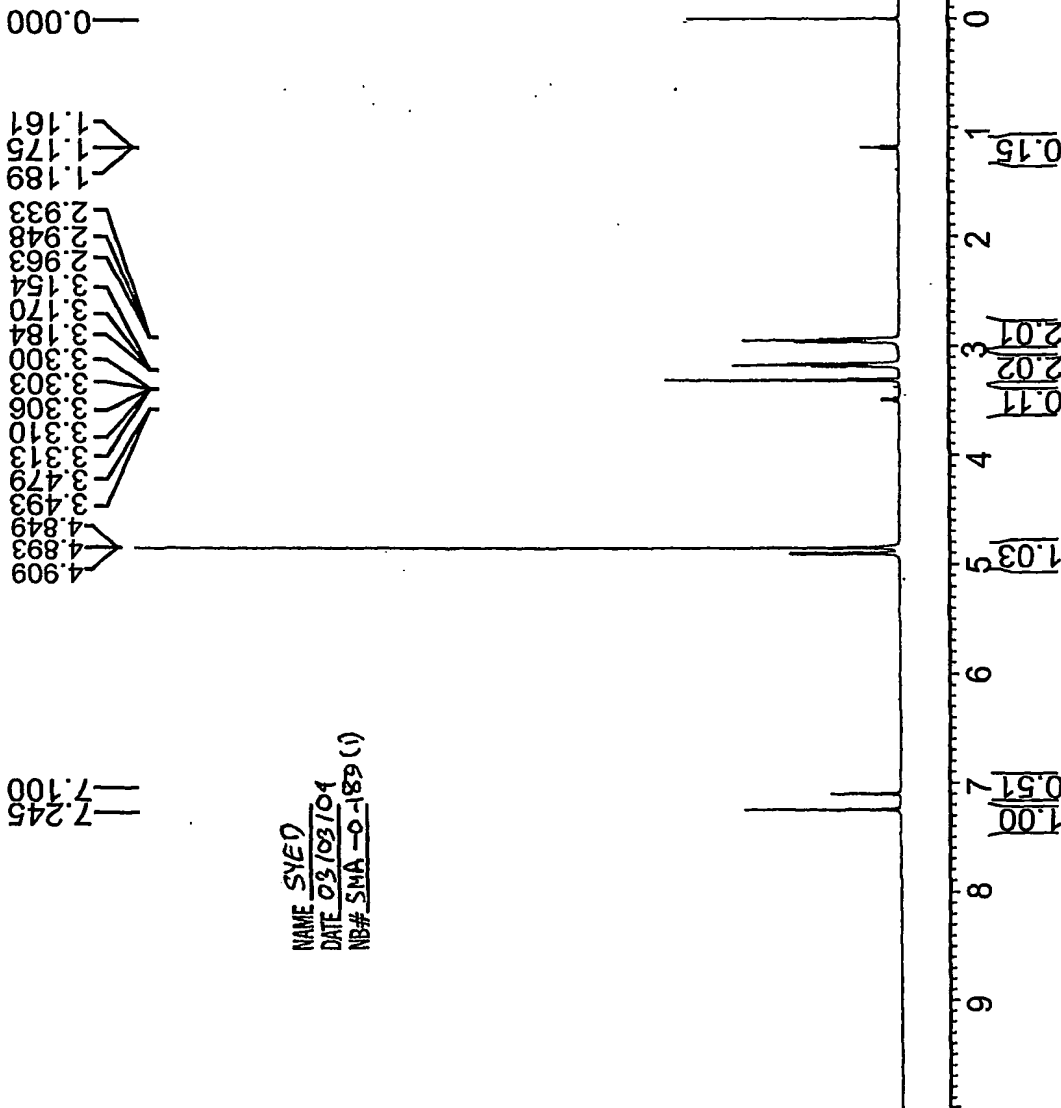
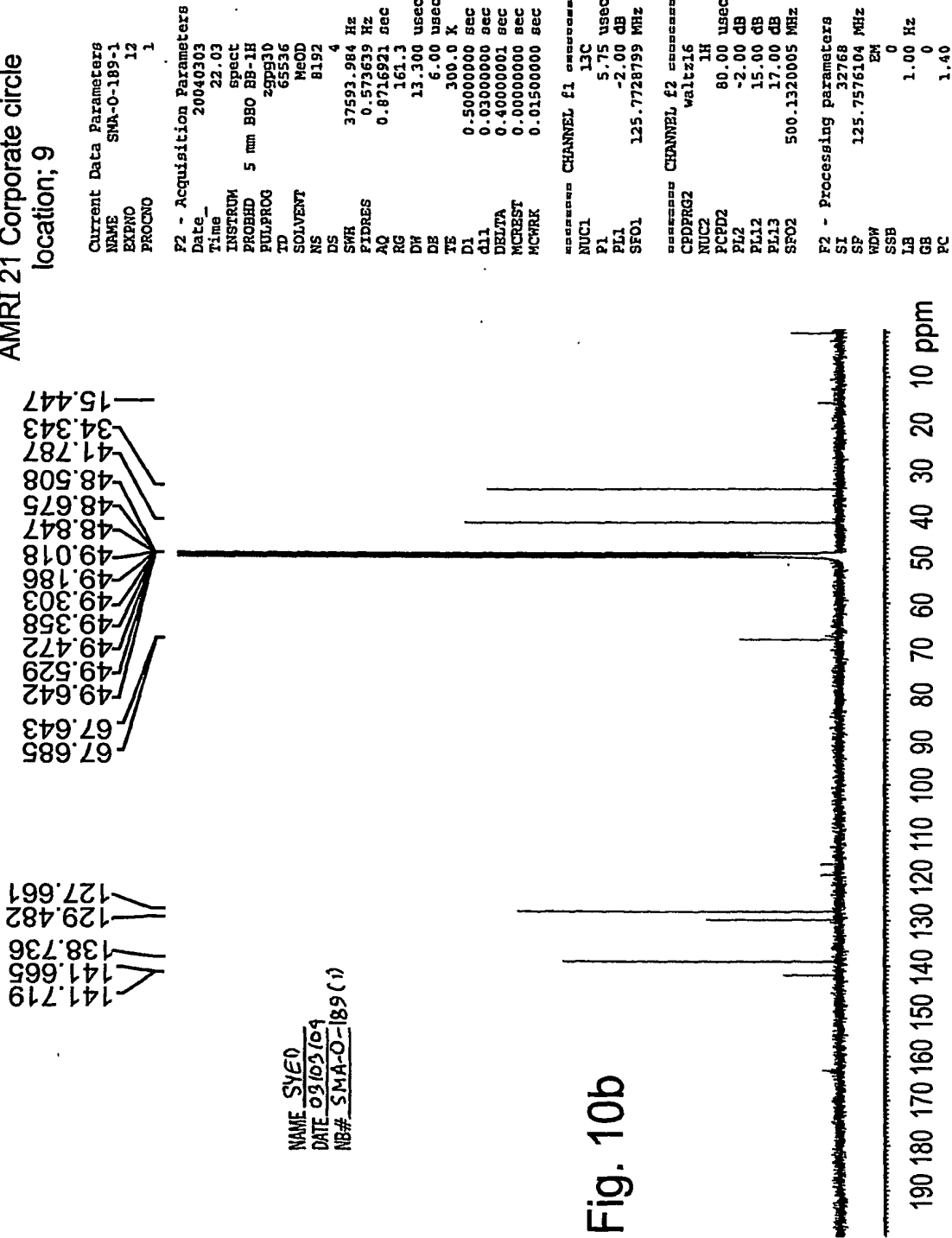


Fig. 10a

AMRI 21 Corporate circle
location; 9



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AMRI 21 Corporate circle location; 9

Current Data Parameters
NAME SMA-O-189-1
EXPNO 11
PROCNO 1

F2 - Acquisition Parameters
Date_ 20040303
Time_ 18.46
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 16
DS 4
SWH 80645.164 Hz
FIDRES 1.230548 Hz
AQ 0.4063794 sec
RG 9195.2
DM 6.200 usec
DE 6.00 usec
TE 300.0 K
D1 2.0000000 sec
d11 0.0300000 sec
DELTA 1.8999998 sec
MCREST 0.0000000 sec
MCWRK 0.0150000 sec

===== CHANNEL f1 =====
NUC1 31P
P1 9.75 usec
PL1 0.00 dB
SFO1 202.4461871 MHz

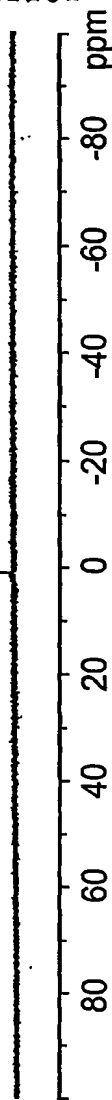
===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -2.00 dB
PL12 15.00 dB
PL13 17.00 dB
SFO2 500.1320005 MHz

F2 - Processing parameters
SI 32768
SF 202.4563100 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

— 1.14

NAME SYED
DATE 02/03/04
NB# SMA-O-189 (1)

Fig. 10c



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Data File: SMA-O-189-103040008_083719
Sample ID: SMA-O-189-1
Acquisition Date: 03/04/04 08:37:28 AM
Run Time (min): 1.49
User Name: S.Mahmood
SMA-O-189-103040008_083719 #12 RT: 0.23 P: + AV: 5 SB: 87-10 14-17 NL: 3.79E6
T:(0,0) + c ESI sid=20.00 Full ms[50.00-1002.00]

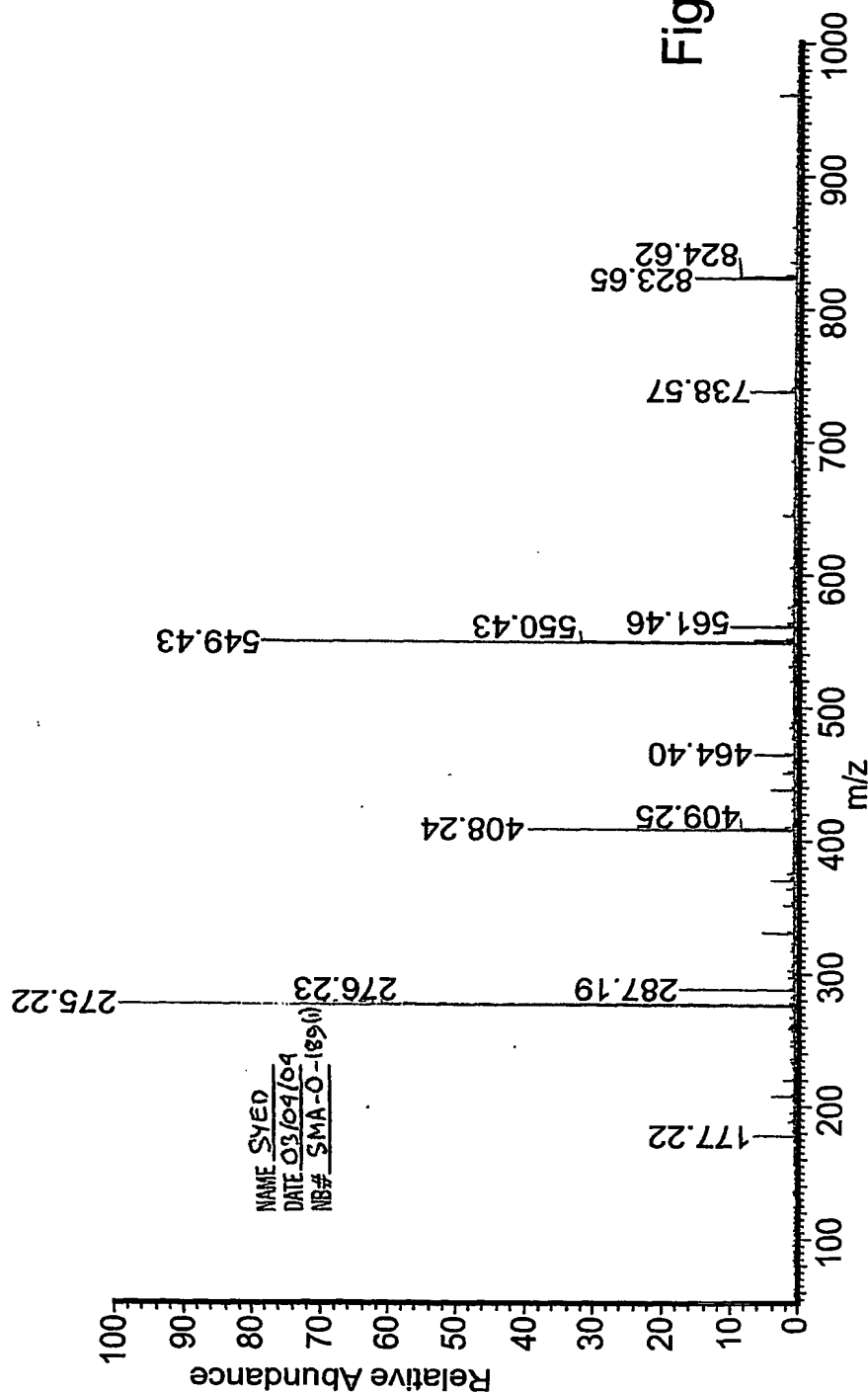


Fig. 10d

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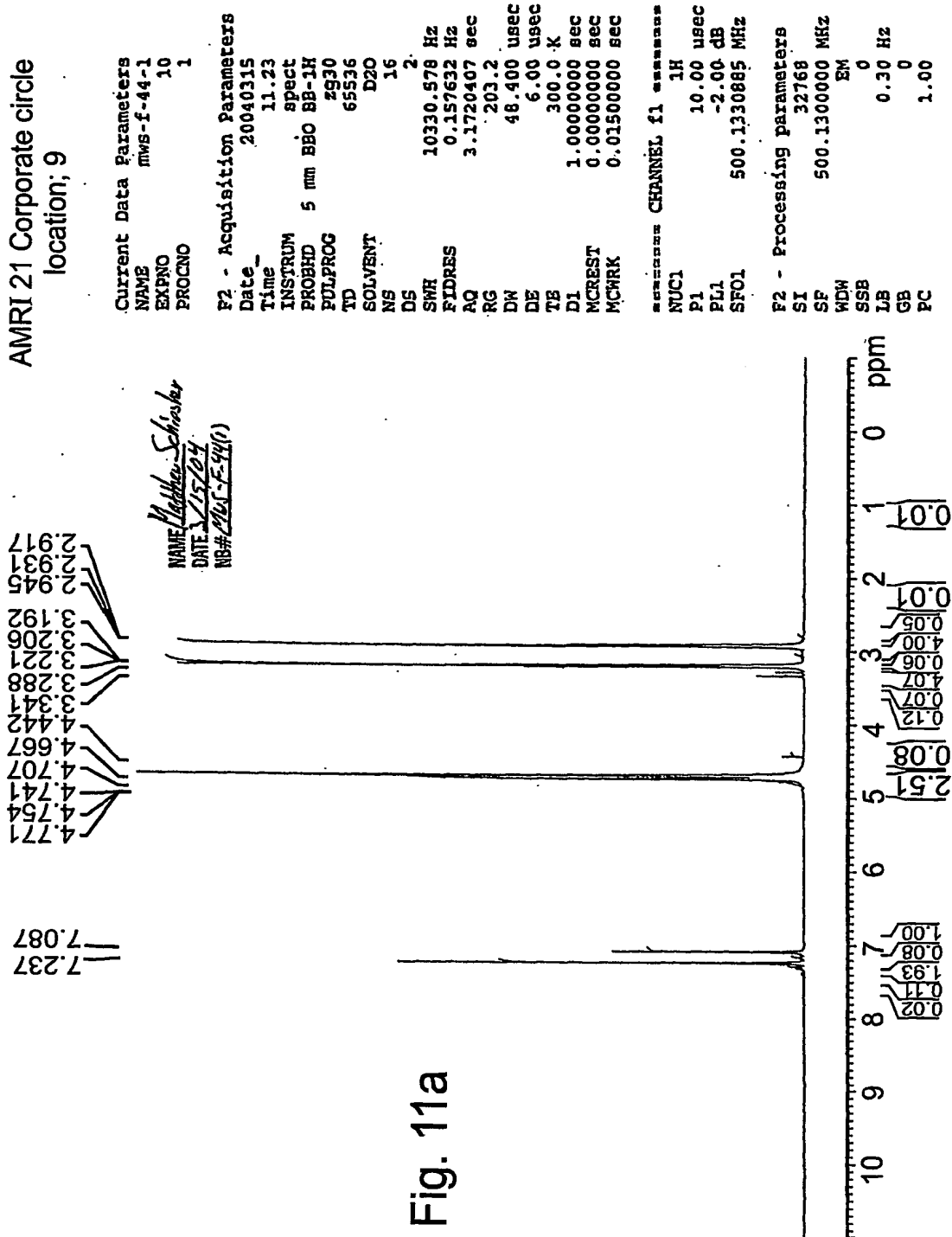
AMRI 21 Corporate circle
location: 9

Fig. 11a

AMRI 21 Corporate circle
location; 9

NAME *M. White-Schmidt*
DATE *3/16/04*
NB # *045-F-48/01*

Current Data Parameters
NAME mmb-1-44-1
EXPNO 20
PROCNO 1

F2 - Acquisition Parameters
Date_ 20040316
Time 0.55
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 10000
DS 4
SWH 37593.984 Hz
FIDRES 0.573639 Hz
AQ 0.8716921 sec
RG 128
DH 13.300 usec
DE 6.00 usec
TE 300.0 K
D1 0.50000000 sec
d11 0.03000000 sec
DELTA 0.40000001 sec
MCREST 0.00000000 sec
MCWRLK 0.01500000 sec

===== CHANNEL f1 =====
NUC1 13C
P1 7.50 usec
PL1 0.00 dB
SFO1 125.7728799 MHz

===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -2.00 dB
PL12 15.50 dB
PL13 17.00 dB
SFO2 500.1320005 MHz

F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

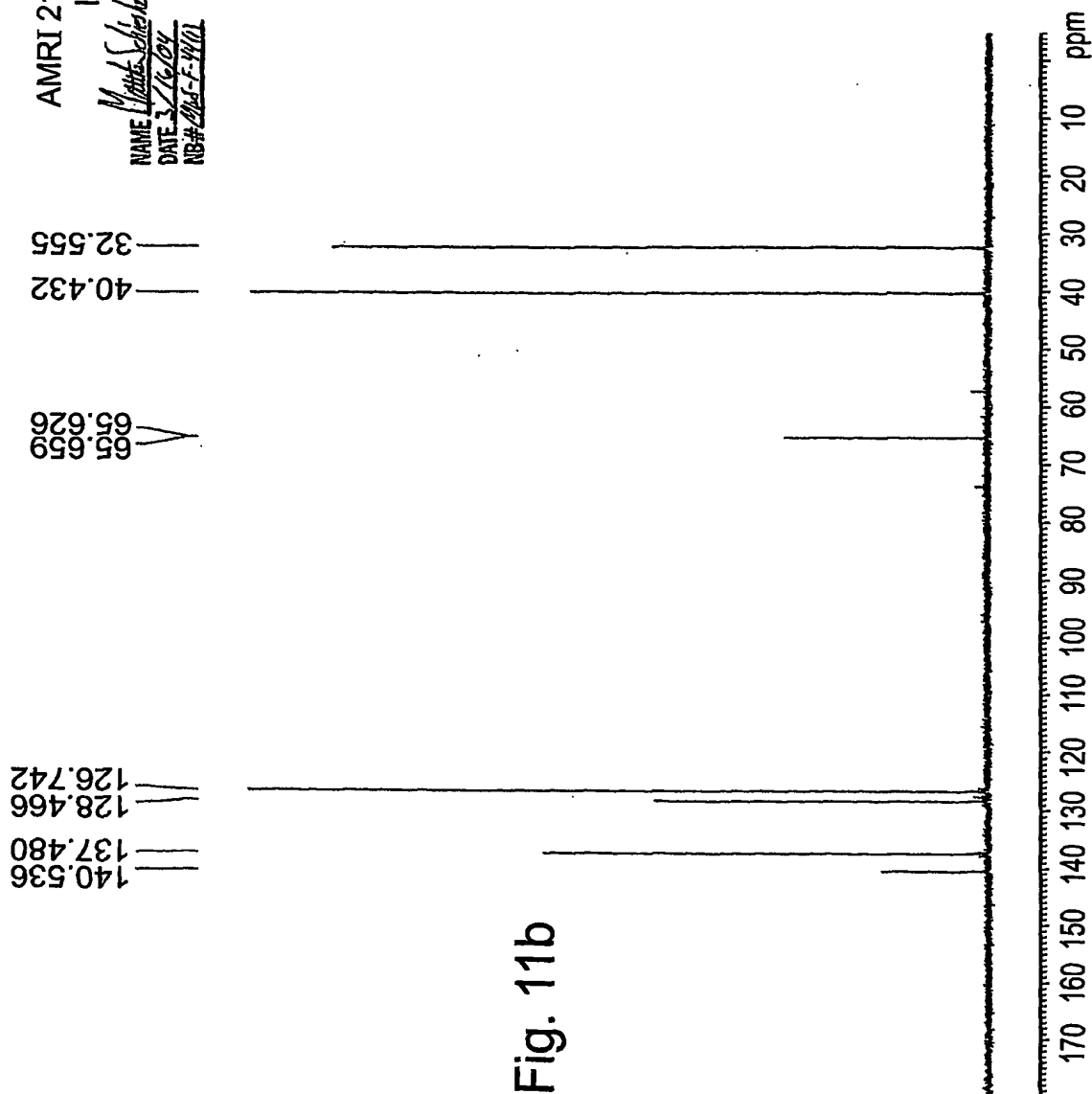


Fig. 11b

AMRI 21 Corporate circle
location; 9

20/24

Current Data Parameters
NAME mws-f-44-1
EXPNO 11
PROCNO 1

F2 - Acquisition Parameters
Date_ 20040315
Time 11.27
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 16
DS 4
SWH 80645.164 Hz
FIDRES 1.230548 Hz
AQ 0.4063794 sec
RG 20642.5
DH 6.200 usec
DE 6.00 usec
TE 300.0 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
MCREST 0.00000000 sec
MCHRG 0.01500000 sec

===== CHANNEL f1 =====
NUC1 31P
P1 9.75 usec
PL1 0.00 dB
SFO1 202.4461871 MHz

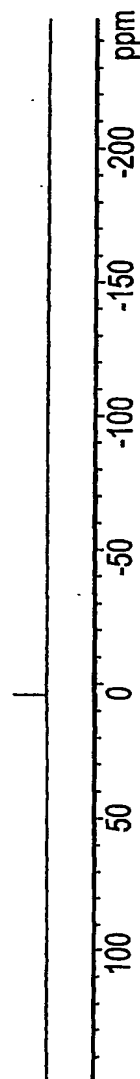
===== CHANNEL f2 =====
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -2.00 dB
PL12 15.50 dB
PL13 17.00 dB
SFO2 500.1320005 MHz

F2 - Processing parameters
SI 32768
SF 202.4563100 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

M. S. J.
NAME MWS-F-44-1
DATE 3/15/04
NB# 2005-F-44(1)

— 3.97

Fig. 11c



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Data File: MWS-F-44-103150037_113834
Sample ID: MWS-F-44-1
Acquisition Date: 03/15/04 11:38:40 AM
Run Time (min): 1.50
User Name: F. Jos
MWS-F-44-103150037_113834 #17 RT: 0.31 P: + AV: 5 SB: 8 12-15 19-22 NL: 8.27E5
T:[0,0] + c ESI sid=20.00 Full ms[50.00-1002.00]

NAME Martin Schuster
DATE 3/15/04
NB # MWS-F-44(1)

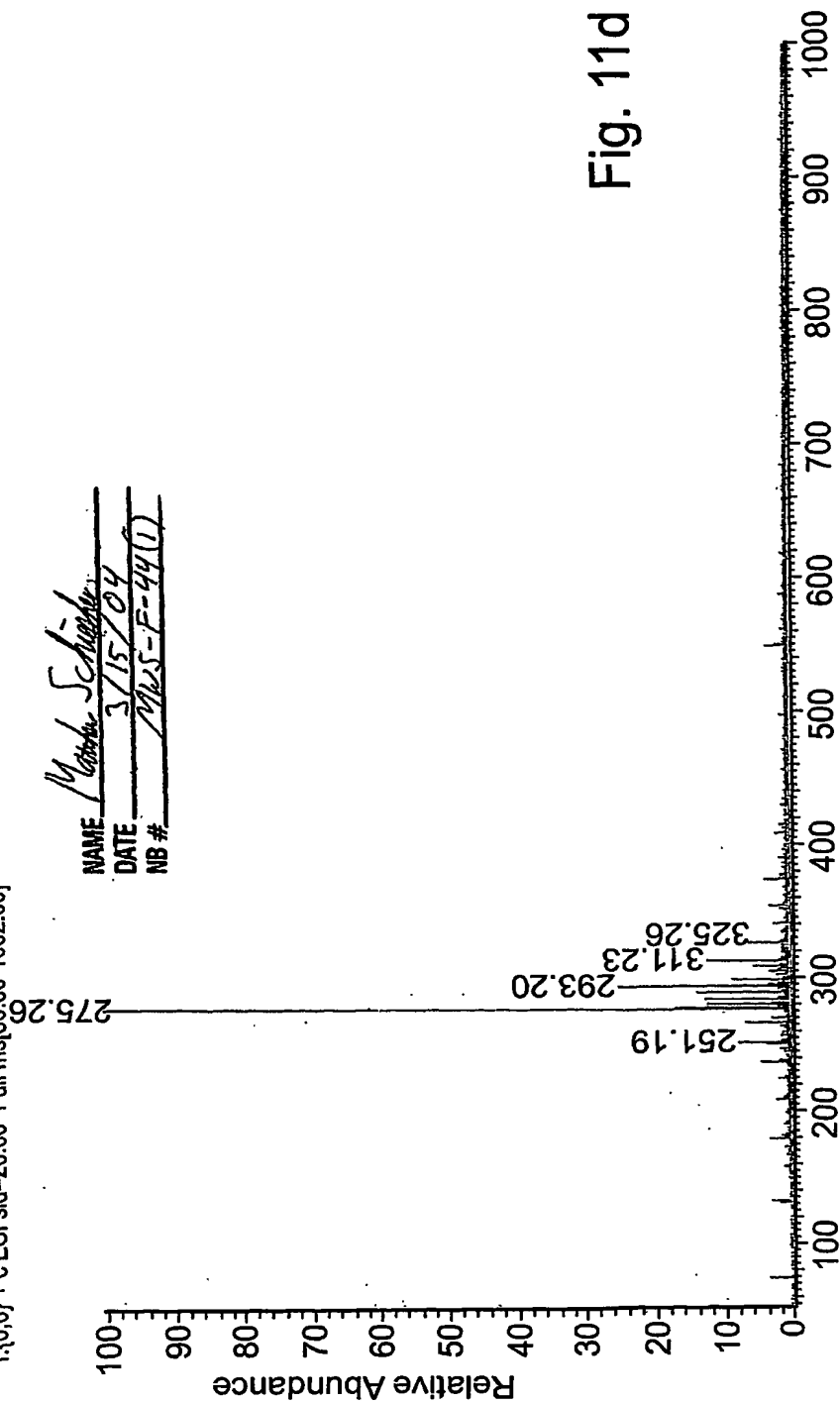


Fig. 11d

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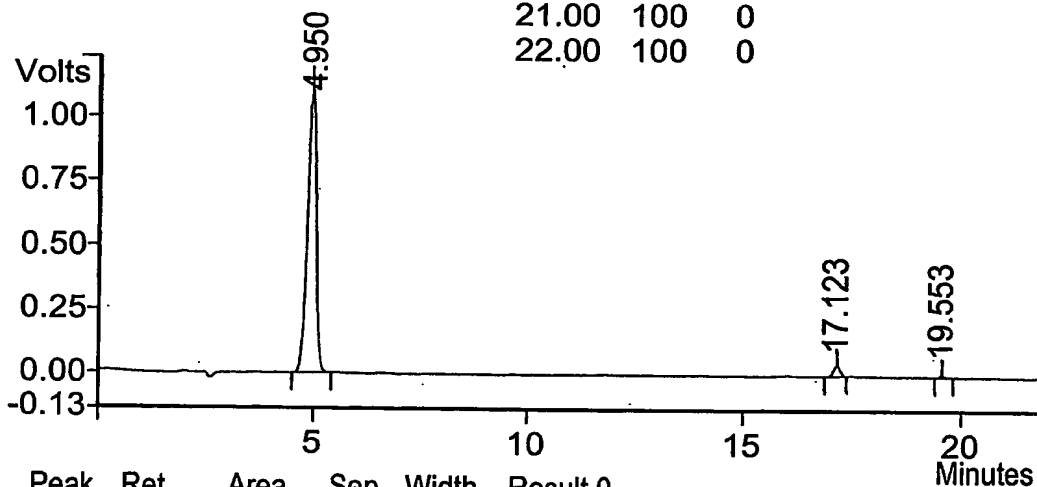
Albany Molecular Research, Inc.
21 Corporate Circle • Albany, NY 12203 USA • Tel 518-444-0279 • Fax 518-444-0281Name : Matt Schiesher
Date : 3/15/04
NB# : MWS-F-44(1)

Channel: 1=INTGR 1 RESULTS
Sample ID: mws-f-44(1)
Injection Date: 03/15/04 10:14:12 AM
Injection Method: c:\star\mws\2009_m6.mth
Peak Measurement: Peak Area
Injection Notes:

Method notes

Luna C8(2), 5u 4.6 x 150 mm
Flow 1.0 ml/min, Monitored @ 220 nm
A-0.1% Aq. TFA B-0.1% TFA in acetonitrile

Time	%A	%B
0.00	100	0
4.00	100	0
20.00	92	8
21.00	100	0
22.00	100	0



Peak No	Ret. Time (min)	Area (counts)	Sep. code	Width 1/2 (sec)	Result 0
1	4.950	15507916	BB	12.7	96.72
2	17.123	448604	BB	10.3	2.80
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Fig. 11e

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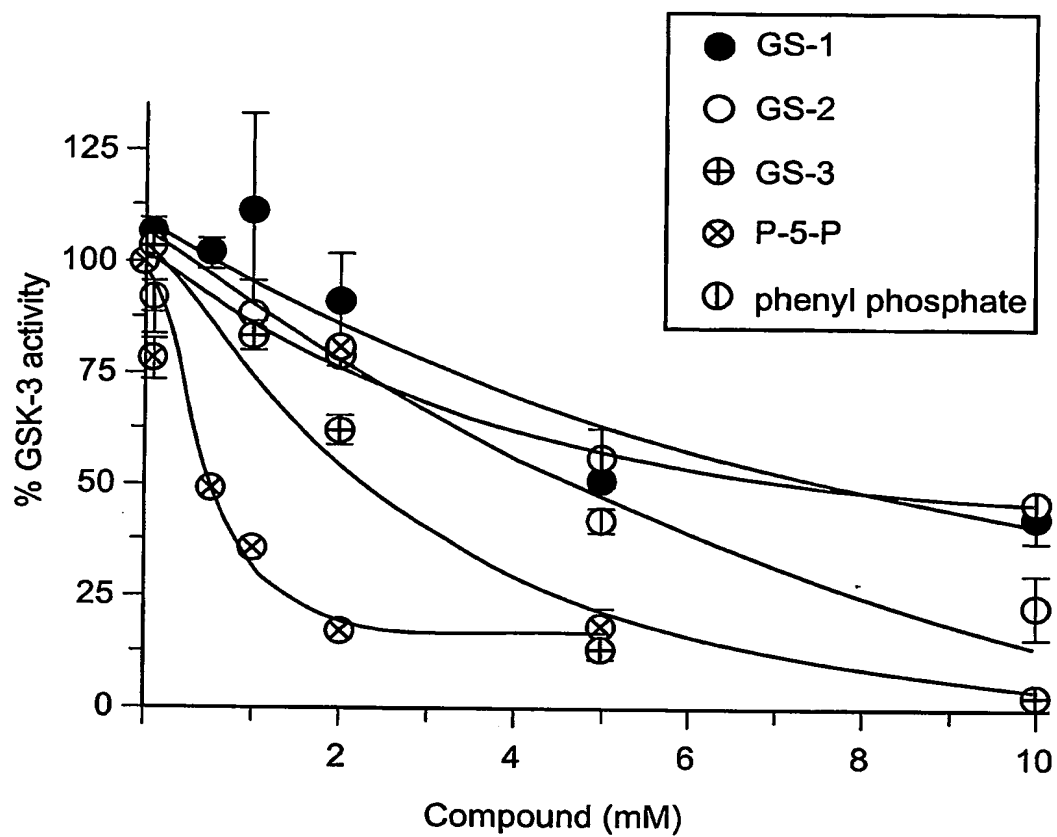


Fig. 12

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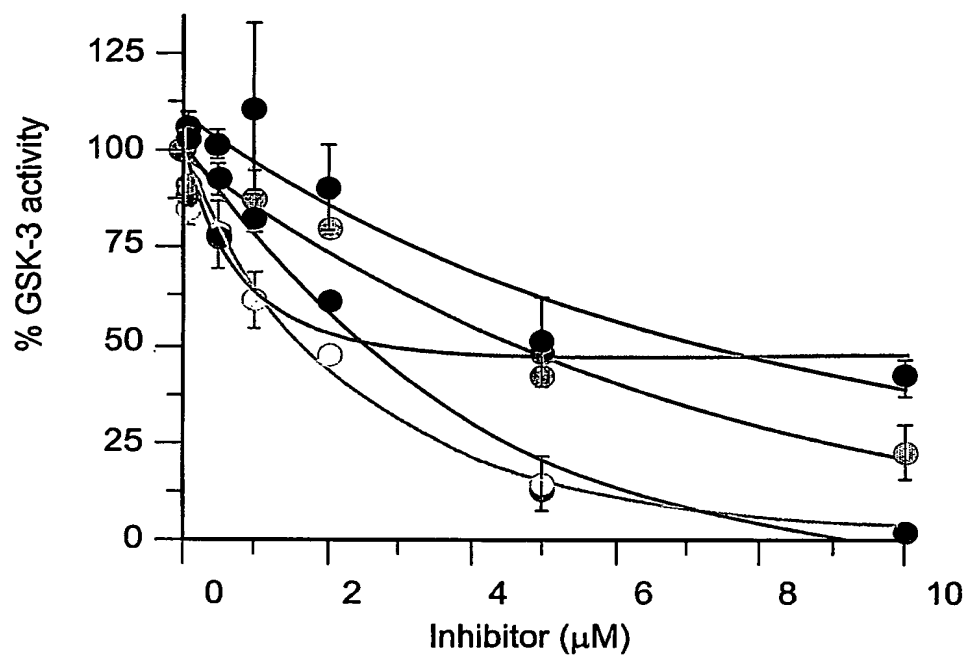


Fig. 13

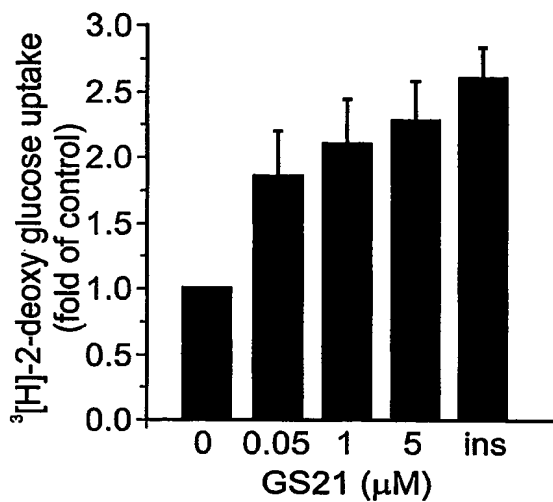


Fig. 14a

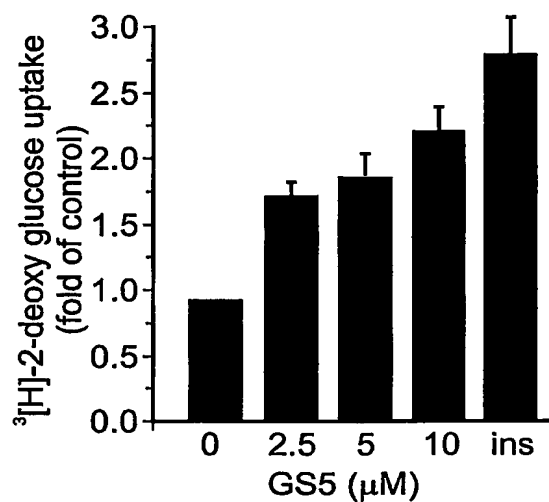


Fig. 14b

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